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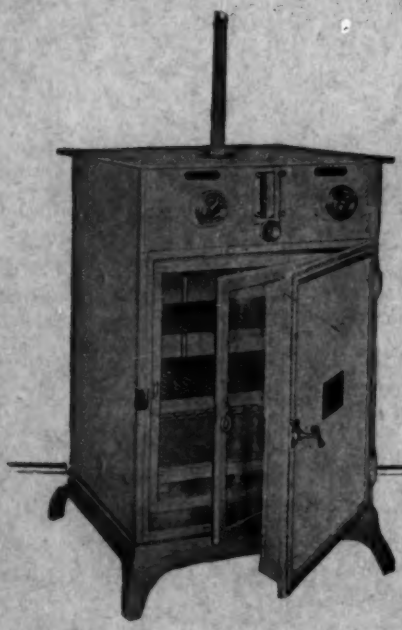

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The Journal of Laboratory and Clinical Medicine

VOL. XIII ST. LOUIS, MO., MAY, 1928 No. 8

CLINICAL AND EXPERIMENTAL

THE ORAL ADMINISTRATION OF POLLEN*

A CLINICAL REPORT

BY J. H. BLACK, M.D., DALLAS, TEXAS

RECENTLY I reported[1] the experimental administration of ragweed pollen by mouth to a ragweed-sensitive individual. That study showed that no ill effect followed the oral use of the pollen and that it was absorbed in appreciable quantities. Herewith I report the oral use of pollen in a group of patients treated during the latter half of 1927, and results are compared with a similar group which was treated by the usual hypodermic method.

These patients, with three exceptions, were sensitive to a grass, a ragweed, or to the two combined. In the three others there was a sensitiveness to careless weed in addition to grass or ragweed. Since the method of treatment and the results obtained were practically the same in the different sensitizations, I have not separated them according to pollens in this report. My results with the ragweed-sensitive patients were as good as those obtained with the grass-sensitive cases. This was true in both the group treated hypodermically and in the one treated by mouth.

My method of hypodermic therapy is briefly as follows: The initial dose depends upon my opinion of the degree of sensitiveness. Usually I begin with 0.1 c.c. of a 1:10,000 dilution. Injections are given daily if the preseasonal period is short or if treatment is coseasonal. Increments in dosage are large until local reactions appear, then increases are made more cautiously. Systemic reactions, of course, call for careful and slow progress. The number of doses given depends entirely upon the rapidity with which the dose can be increased and the amount to which the dose must be carried to effect relief. The final dose is considered that amount which will insure complete protection. This, of course, varies widely with different people and, in some cases, was not attained. As the large doses were reached, the interval was usually increased to forty-eight hours and the "protecting dose" was repeated at weekly intervals if necessary. The average protecting dose in this series was 0.6 c.c. of a 1:20 dilution. All our extracts were made with 46 per cent glycerin in 7 per cent sodium chloride as recommended by Stier.[2] Dilutions were made with carbolized normal saline.

The oral administration of pollen was practically coseasonal. Since it was learned that protecting doses could be reached quickly, it was unnecessary to begin treatment weeks before the season. Patients who presented themselves before the onset of the season were told to report for treatment on appearance of the first symptoms. Those appearing after the development of symptoms were started on treatment at once. The extract was dispensed in dropper-stoppered bottles. The initial dose was 10 drops of a 1:20 extract. Each succeeding dose was increased by 10 drops until 60 drops were being taken at a dose. Doses were taken three times daily so that the dose of 60 drops was reached within forty-eight hours. The extract was dropped into a glassful of water, milk, or other beverage, stirred well and drunk. The usual instruction was to take it before meals, but occasional individuals found some nausea occurring when the stomach was empty, which could be prevented by taking the pollen after meals. I was unable to find any difference in the results dependent upon the presence or absence of food in the digestive tract. The dose of 60 drops was made arbitrarily the maximum after I had found that patients who did not show any protection at that dose did not do any better if the dosage was carried higher. Under other conditions, such as different pollen environment and the use of different extracts, the dosage required to protect might be entirely different. That there is a maximum dosage, beyond which it is useless to go, seems apparent. We found no appreciable difference in the dosage of children and adults. In those patients who secured protection by 60-drop doses, this dose was maintained for a few days and then lowered five drops per day until the minimum was reached as shown by the reappearance of symptoms. There was no uniformity in this respect. A few patients remained well on doses of 20 drops once a day. Others required more, and many required the 60-drop doses three times daily. The variation in this respect is quite similar to that which is found in the protecting dose of hypodermic treatment.

The cases reported are unselected and represent all the cases treated during the second half of 1927 except those whose treatment is still in progress, those who, for one reason or another, did not carry treatment far enough to determine results, and those from whom we have been unable to get reports. The percentage of improvement shown in Table I is that claimed by the patient, checked whenever possible by my own observation, and is believed to be a conservative statement. Under the heading "75 per cent" are grouped those patients who could claim 75 per cent or more of improvement but could not be listed as 100 per cent cases. Likewise, the 50 per cent group is made up of those whose percentage of improvement ranged from 50 per cent to 70 per cent. I believe this is a conservative method of presenting the figures and that, as presented, they do not overestimate the benefit obtained.

Table I presents the number of cases and the percentage of the total number of both hay fever and asthma patients obtaining the different degrees of improvement. The total number of hay fever patients treated orally (73) is slightly greater than the number receiving hypodermic therapy (61), but the number of asthma patients treated orally (18) is small compared with the number treated hypodermically (32). The difference in the number of asthma

TABLE I

RESULT	HAY FEVER				ASTHMA			
	ORAL		HYPODERMIC		ORAL		HYPODERMIC	
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
100%	13	17.80	15	24.60	6	33.3	16	50.0
75%	25	34.25	33	54.10	6	33.3	8	25.0
50%	15	20.55	9	14.75	2	11.1	7	21.85
25%	5	6.85	0	0.0	0	0.0	1	3.15
0%	15	20.55	4	6.55	4	22.2	0	0.0

patients treated by the two methods was not due to the difference in the results expected but to the fact that pollen therapy by mouth over long periods of time is usually quite expensive.

It is apparent that the results with hypodermic therapy are better than those with pollen given orally. This is evidenced not only by the larger percentage who received substantial benefit from treatment but also by the smaller number who got no appreciable results from treatment. Of those obtaining 75 per cent or more of improvement—which I consider quite satisfactory—there is a definite difference in favor of hypodermic therapy. There were many more complete failures with oral treatment.

In 13 cases of hay fever I changed from oral to hypodermic treatment because of gastrointestinal symptoms or because they were getting too little improvement. Six cases of asthma were similarly changed. Three cases of hay fever and one of asthma were changed from hypodermic to oral therapy because of violent systemic reactions from the former method. The percentage of improvement in each type of treatment is shown in Table II. It is evident from these figures that a failure to get results by oral treatment does not preclude the possibility of controlling the case by hypodermic therapy and that some patients in whom severe reactions prevent satisfactory hypodermic treatment a change to oral administration may be of benefit.

TABLE II

CASES CHANGED FROM ONE METHOD TO THE OTHER

ORAL TO HYPODERMIC				HYPODERMIC TO ORAL			
HAY FEVER		ASTHMA		HAY FEVER		ASTHMA	
ORAL	HYPO	ORAL	HYPO	HYPO	ORAL	HYPO	ORAL
0	50	0	0	90	90	60	75
0	60	0	0	75	90		
0	75	0	75	75	90		
0	100	75	75				
25	75	75	75				
25	90	75	90				
25	90						
50	50						
50	80						
50	80						
75	75						
75	75						
75	90						

*The figures represent the percentage of improvement received.

Six of the hay fever patients changed from oral to hypodermic therapy were changed because of nausea, diarrhea, and abdominal distress. There were a few others who had slight nausea, but these were relieved by changing

the time of dosage from before to after meals. In all these patients the abdominal symptoms came on within from twenty-four to forty-eight hours after oral treatment was begun and persisted until it was stopped. None of the usual medicinal agents gave any relief, but symptoms disappeared promptly on discontinuance of treatment. These patients were then put on hypodermic treatment with no recurrence of symptoms. The natural assumption, I think, is that these individuals have a sensitization of the mucosa of their digestive tract as well as of their respiratory tract, but I have no other evidence to substantiate such a belief. It is interesting to note—although it may have no significance—that all of these six patients were made ill by *ragweed* pollen. It is possible that these symptoms might be avoided by giving the pollen more frequently in smaller doses, but I have not yet done this.

In changing from the oral to the hypodermic method, an interesting and important finding was made. In those patients who had received a considerable degree of protection by oral treatment, it was possible to begin hypodermic treatment at a high dose; usually as much as 0.1 c.c. of a 1:20 dilution could be given safely. However, in those who got little or no protection from oral treatment, hypodermic treatment had to be started at the beginning as though they had had no treatment at all. Failure to recognize this fact caused some severe systemic reactions. These patients, after taking large quantities of pollen by mouth, were apparently as sensitive to it as formerly. In these cases I have been unable to find any of the "atopen" in the blood stream, and I believe that, for some unknown reason, there is little or no absorption of the pollen.

I have always had a great deal of difficulty in carrying up the hypodermic dosage of careless weed cases. Systemic reactions are frequent and severe in spite of all precautions, and carrying a patient to his "protecting dose" is a prolonged and difficult procedure. In the three cases reported in which careless weed was a factor, I have adopted the plan of giving the careless weed by mouth even though the other pollen to which the patient is sensitive may be given hypodermically. In these three cases protection has been secured with a very welcome absence of systemic reactions.

None of the patients receiving pollen orally had any asthma, hay fever, or urticaria as a result of treatment. Early in this work several patients received as much as 300 drops—equivalent to approximately 15 c.c.—of the 1:20 extract each day for three or four days. In none of them was there any evidence of symptoms, such as I see frequently from hypodermic therapy. In contrast to this six hay fever patients and one of asthma, treated hypodermically, had violent systemic reactions. Three of these patients refused further treatment because of fear of another reaction.

The oral administration of pollen has certain definite advantages over hypodermic therapy.

First, treatment does not require a prolonged preseasonal period but is entirely coseasonal. In this respect it is of distinct advantage in those patients who present themselves for treatment after the season has begun.

Second, patients do not have to report to the physician for each dose of pollen but can carry on the treatment under his direction at home. This saves

much time for both physician and patient; makes it possible to care for a much larger number of patients, and permits the treatment of out-of-town patients and travelling men without the fear of reactions from improper or excessive dosage.

Third, the extract diluted largely in water, milk, or other beverage is almost tasteless and children take it without objection. The pain, which is such an objectionable accompaniment of the injection of the glycerin-saline extracts, is avoided.

Fourth, systemic reactions do not occur. This is in marked contrast to the rather frequent occurrence of reactions in those treated hypodermically. It is a source of much satisfaction to both patient and physician.

The disadvantages of oral treatment are:

First, the material, in the amounts usually used, is expensive. This becomes a matter of importance in prolonged administration and in the use of those pollens whose cost is high. I have not attempted to use this method in many cases of asthma because the continued use of large doses was too expensive for most patients. I have used it frequently to get the patient under control promptly and then changed to hypodermic dosage to maintain protection. This is an excellent, time-saving plan in those patients who respond to oral therapy. I have not attempted oral treatment with such pollens as mountain cedar, because its cost is prohibitive. Timothy grass and the ragweed pollens may be bought in quantities at a low price and lend themselves well to this manner of use.

Second, a small percentage of patients cannot take pollen by mouth because of nausea and abdominal pain. It is possible that they might be able to take it if the dosage were divided into small amounts and taken more frequently, but I have not tried this method of usage.

Third, the percentage of those securing satisfactory protection is less than with hypodermic treatment, and the percentage of complete failures is considerably higher. The figures might be somewhat different if the series were larger, but I believe the conclusion stated is justified. If some method might be devised by which one could determine whether a given patient would respond to oral treatment without waiting for a clinical test at the onset of the season, it would be a most desirable thing. At present, if oral treatment is begun at the onset of the season and fails to protect the patient, it makes it necessary to begin coseasonal hypodermic therapy, the patient suffers unnecessarily, and the results are not so good. At present, there is no method available for eliciting this information.

SUMMARY

A comparison is made of results obtained by oral and by hypodermic pollen therapy. The conclusion seems justified that oral treatment has certain definite advantages but that satisfactory protection is secured less often.

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1405 Medical Arts Building.

PRACTICAL CONSIDERATIONS OF METAPLASIA IN NEOPLASTIC DISEASES*

By Harold D. Caylor, M.D., Rochester, Minn.

THE term metaplasia may perhaps be given properly to the transition of one tissue into another of a related kind or to the formation of different tissues from a common parent cell. By certain observers metaplasia is defined as the production of tissue by cells that usually produce a different type of tissue. This change of one type of tissue to another has been seen in many organs, including the skin, gall bladder, stomach, and uterus. Practically, metaplasia has been of little general interest and importance. It has been well described by Lubarsch and others. The present observations concern meta-

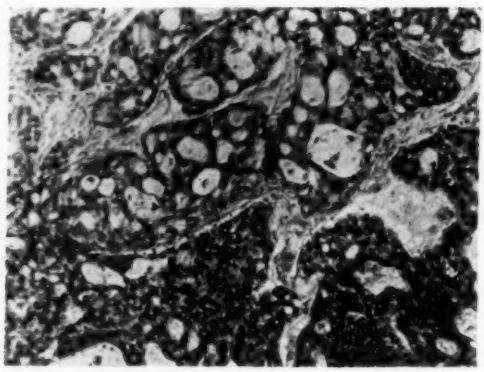

Fig. 1.—Adenocarcinoma of the breast. (x120)

plasia of columnar epithelium to squamous epithelium, and its practical applications to the diagnosis and treatment of neoplasms.

Lubarsch,[3] in referring to all changes of cell form in general, used the term "alloplasie" or "heteroplasie" and, under this heading, outlined three main groups: (1) pseudometaplasia; (2) metaplasia, and (3) undifferentiation. Pseudometaplasia he described as "a simple change of the form of cells, metaplasia as a genuine change of a specific cell or tissue to another of similar kind, and undifferentiation as the change of sharply differentiated cells into undifferentiated cells." The last group was divided into two types: physiologic undifferentiation by indirect nuclear division, which was the ordinary mode of cell division, and pathologic undifferentiation which was called by von Hansemann "anaplasia" and by Beneke "kataplasia." Present interest is in the second group, metaplasia.

*Submitted for publication, November 23, 1927.
Section on Surgical Pathology, Mayo Clinic, Rochester, Minnesota.

EXAMPLES OF METAPLASIA

Squamous-cell epithelioma developing in the gall bladder is an excellent example of metaplasia.[2] Normally there is no squamous epithelium in the gall bladder or in any of the adjacent organs, and it is by assuming that this tumor arose partly because of the change of the tissue by metaplasia that its origin can be explained. Another example of metaplasia in epithelial tumors is a squamous-cell epithelioma developing in malignant papillary cystadenoma of the ovary.[4] A diagnosis of metaplasia in a neoplasm can be safely made only

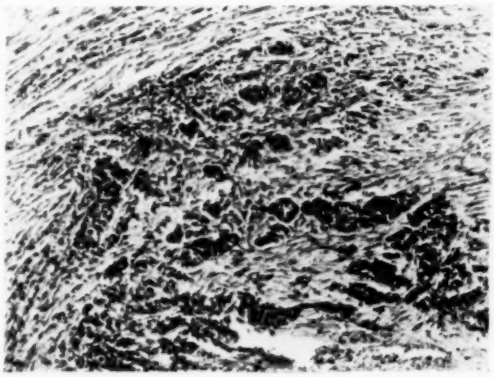

Fig. 2.—Metastatic adenocarcinoma in an axillary lymph node. (x120)

Fig. 3.—Squamous-cell epithelioma in an axillary lymph node. (x120)

after careful examination of the body has failed to reveal another tumor. Two different but related neoplastic disorders can be present in the same portion of the body at the same time. Two kinds of metastatic tumors may occur in lymph nodes from two primary malignant neoplasms elsewhere in the organism. To determine whether two related masses present in lymph nodes are due to multiple lesions elsewhere, or to metaplasia of one type of tumor to another, careful search must be made for distant primary lesions.

I have observed areas of metastatic squamous-cell epithelioma in the axillary lymph nodes in cases of adenocarcinoma of the breast. The growth in the breast and in most of the involved lymph nodes was typical adeno-

Fig. 4.—Cervical polyp containing areas of squamous epithelium. (x60)

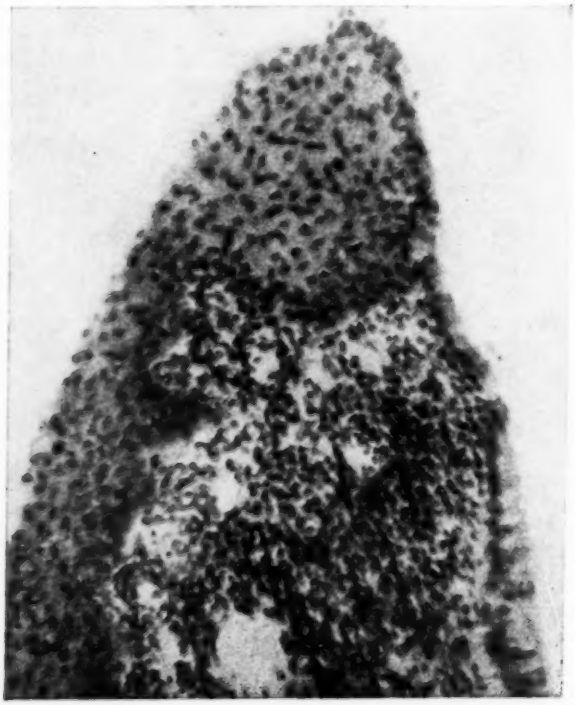

Fig. 5.—Portion of a cervical polyp covered in part by squamous epithelium and in part by columnar epithelium. (x200)

carcinoma (Figs. 1, 2, and 3). Careful examination of the excised tissue did not reveal a primary epithelioma, and evidence of the presence of any other neoplasm could not be discovered at physical examination. This is probably an example of metaplasia in metastasis from carcinoma of the breast, although one cannot be positive of metaplasia unless there has been complete examination of the body to make certain that no obscure primary malignant tumor is present.

Polyps in the cervical canal of the uterus are rarely malignant and are usually covered with columnar epithelium. I have examined cervical polyps which were covered with columnar epithelium in some areas and with squamous epithelium in others with the formation of epithelial pearls (Figs. 4 and 5). When tissue of this character appears at operation, two questions must be decided. Is the growth a benign cervical polyp with metaplasia of columnar epithelium to squamous epithelium, or is it a squamous-cell epithelioma developing in a cervical polyp? If the first diagnosis is made, the condition is benign and of little consequence, and removal of the polyp would be sufficient. If the second diagnosis is made, the operative procedure should be radical and the prognosis guarded. Careful microscopic examination of the tissue in general and of the particular cells reveals no evidence of malignant disease in these polyps and indicates that they are examples of metaplasia of columnar epithelium to squamous epithelium and that the lesion is benign.

These cases illustrate some important practical considerations of metaplasia in tissues that sometimes must be considered in the diagnosis of tumors and in the subsequent treatment to be employed.

SUMMARY

The change from one type of malignant tissue to another of a related kind gives rise to the question: Is this metaplasia or is there more than one primary malignant tumor present? In benign tumors, such as cervical polyps, the covering epithelium, by means of metaplastic changes, may be altered to a different type of related tissue which may simulate a malignant growth although the lesion is actually benign.

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FURTHER STUDIES ON THE ORGANISM WHICH PRODUCES SPECIFIC LESIONS IN THE STOMACH AND DUODENUM AND AN INTRADERMAL TEST, RELATIVE TO CHRONIC AND LATENT INFECTIONS*

By Albert Hoffmann,† Anaheim, California

IN A PREVIOUS paper[1] I described an organism (hereinafter called Bacillus Hoffmanni) which produced with great regularity, either duodenitis, or ulcer, or both, in the guinea pig, and if injected intramuscularly or if the animal was left to develop a chronic state of the disease, myositis. The object of the present paper is to report what further studies of this organism have revealed.

An organism which can be isolated from man, only with great difficulty, but which, if once isolated in a pure state, grows on the ordinary culture medium very readily, remaining alive for a period of two years without being subcultured, and yet, retaining its specificity, is surely worthy of serious investigation.

A culture grown for nine months in Hiss' serum-water had, aside from a heavy precipitate, a translucent supernatant fluid which upon subculturing into broth media and left at room temperature for a period of twelve months, developed small vesicles the size of lentils which suggested a fungoid growth. Upon microscopic examination only clumps of bacteria, some of them resembling fatty acid crystals, could be seen. Stained smears revealed the same gram-negative organism with which the medium was inoculated.

Of the above culture 2 c.c. were inoculated intracardiac into a large guinea pig and another received 3 c.c. intraabdominally. Both animals developed slight symptoms of toxemia after seven hours and both were chloroformed. The one injected into the heart had a deep crater ulcer close to the pylorus and numerous smaller erosions at the cardiac end of the stomach from which blood was oozing. The second animal's stomach was inflamed along the greater curvature and slight duodenitis was present.

This indicates that the twenty-one-months-old culture has lost nothing of its virulence and its elective affinity for the gastric mucosa. The culture was subcultured without passing through an animal. The broth medium within twenty-four hours was cloudy and had a heavy pellicle which at first suggested a contamination. Never before had such a heavy pellicle formed in such a brief space of time. Upon examination nothing could be found except the original organism. The heavy pellicle was shaken down and another one was formed during the next twenty-four hours.

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†The writer is associated with the Glendale Medical Group, Glendale, Calif., since February, 1927.

[1]American Journal of the Medical Sciences, August, 1925, No. 2, clxx, 212.

After growing the organism for five days in broth medium 4 c.c. were injected intraabdominally at 9:30 A.M., and another guinea pig received the same quantity of a culture grown for twenty-four hours only. Toxemia developed within one hour. The first animal was visibly worse than the second; the latter, however, became very ill later in the afternoon. The five-day-old-culture animal died at 4 P.M. The other animal, although still alive at 5:30 P.M. was very sick and was chloroformed.

Necropsy.—There were no lesions in the intestines of the first guinea pig. The stomach was hemorrhagic throughout, having deep crater-like erosions ¼ by ¼ of an inch containing blood clots which had partly turned brown in color. In this instance one could sharply differentiate the duodenum from the jejunum; the former being severely inflamed while the latter showed not a sign of inflammatory reaction. The pancreas, too, was hemorrhagic. No free HCl was present in the stomach contents; however, a strong alkaline odor was noticeable. The second animal which had been injected with the twenty-four-hour culture had similar lesions, but in a milder form. The stomach was not filled with blood clots but had distinct signs of inflammation affecting the serous coat and mucosa, the latter having numerous small hemorrhagic areas scattered along the greater curvature.

This is another demonstration of the virulence of this organism, brought about simply by subculturing.

The predisposition of the gastric mucosa to peptic ulcer when a large number of the organisms is injected might possibly be explained by the sudden resultant alkalosis. The high degree of alkalinity of the culture medium is doubtless responsible for such a condition. The instantaneous neutralization of the stomach contents, and the destructive action of the alkali on the mucosa, are seemingly enough to break down the protective barrier, allowing the bacteria injected with the already present microorganisms and enzymes to do their destructive work. If the animal survives, chronic crater ulcers develop, some of which will heal. This was observed in animals which received small doses. One of them, after being injected, was in perfect health after six weeks. Necropsy revealed one small active crater ulcer and many healed ulcers. These were seen as white fibroid areas on the outside of the peritoneal coat. At times there has been observed in the guinea pig (after giving an adequate intracardiac dose of the organism) the whole stomach filled with mucus and blood, having a nauseous and alkaline odor. All there remained, after inverting the stomach, was the muscular and peritoneal coat. From this material, Bacillus Hoffmanni was isolated in pure culture by simply seeding some of the mucous upon Russell's double sugar agar.

The individuals suffering from infected foci which harbor this organism in conjunction with others capable of absorbing its toxins and thus acting as intermediary hosts may develop a complex variety of symptoms.

A guinea pig injected with the specific organism showed symptoms of toxemia within half an hour after receiving 4 c.c. intraabdominally. Seven hours later the animal was chloroformed. From the crater ulcer which was situated close to the pylorus, material was taken and grown in glucose-brain

medium.[2] It was also directly seeded upon eosin-methylene-blue agar plates. The cultures were examined twenty-four hours later. A pure growth of gram-positive streptococci were found in the glucose brain medium, while the agar plates revealed small colonies which upon examination proved to be the same gram-negative organism with which the animal was inoculated (in other words Bacillus Hoffmanni), but no streptococcus could be found, nor any other gram-positive organism demonstrated. Upon careful examination of the glucose brain medium there were noted some gram-negative cocci and other pleomorphic forms which were, however, attached to the gram-positive streptococci and could hardly be differentiated from them. On the second and fifth day the morphologic characteristics of the streptococci did not change.

Four c.c. of the above forty-eight-hours-old streptococcus culture were inoculated into a guinea pig weighing 250 grams. The animal was chloroformed after three days. No symptoms had developed.

Necropsy.—The duodenum was inflamed and a small ulcer at the cap could be seen. The stomach had two minute ulcers at the greater curvature, a large area of the serous coat was inflamed and the stomach on opening, contained gas, and a slight amount of mucus which was adherent to the bulk of the food contents.

A second animal was injected with a five-day-old culture, an intracardiac injection of 2 c.c. being given. The animal was apparently well after twenty-four hours, when it was chloroformed. Necropsy revealed similar results as in the guinea pig referred to above as having been chloroformed seven hours after injection. Lesions were expected (since I believe that Bacillus Hoffmanni was present in the streptococcus culture), but they were mild. It is interesting to note that Bacillus Hoffmanni, which grows in glucose brain medium luxuriantly and produces lesions in the rabbit (as described in the first paper), was found wanting in ability to reproduce itself. What physiochemic relation exists between this streptococcus and bacillus is not clear to me. That the streptococcus acts as a possible repressor, perhaps restricting the virulence of the organism in a being suffering from such an infective process is not unlikely, although it remains still a problem which deserves serious consideration and investigation. It has been observed repeatedly that the streptococcus was present in apparently pure culture if grown in glucose brain medium, whether the material was taken from a focus of infection or from a peptic ulcer, but only with difficulty was Bacillus Hoffmanni isolated from such material. Even when making intramuscular injections with the pure organism producing the typical lesions, I was unable at times to reisolate it from the muscle, but upon careful inspection of smears made from the bone marrow I was able to demonstrate the organism microscopically and actually grow it on the ordinary media, reproducing the specific lesions again in subsequent inoculated animals.

Up to the present time I have been able, although only with great diffi-

[2]Med. Clinics, North America. Mayo Clinic, September, 1921.

culty, to isolate several strains directly either from foci of infection, such as the nose and the tonsils, or from the stomach contents of individuals suffering from this type of infection.

SKIN TEST

An organism possessing such striking pathogenicity for the guinea pig may play an important rôle in chronic and latent infections.

Even before publication of the first paper, I was curious to know whether the organism was seriously connected with infective processes in man or was merely of secondary importance. After much deliberation it was decided to try agglutination and precipitation tests. These were abandoned, because the results were not striking enough to warrant serious investigation. At last, resort was had to the skin test, from which no positive results whatever were expected. Should, however, the human skin be able to record a positive reaction in those individuals afflicted with such an infection, and a negative reaction in those affected with different ailments or in good health, then, and only then, might one be a little more certain of the organism's pathogenicity in relation to man.

The first test was made on a person who had had nasal catarrh for the last fifteen years and had had four operations and local treatment with but slight relief. For the test, there was used a 1 per cent suspension of a seventy-two-hour culture in isotonic saline solution, to which was added 0.5 per cent phenol. Generally one can give up to 0.1 c.c. of a 1 per cent bacterial suspension intradermally without serious effects, but this does not hold with this organism. The patient was given 0.05 c.c. intradermally. After one-half hour a white wheal developed at the point of inoculation but nothing else to justify any kind of conclusion to be drawn. The following day the patient was very ill and had to remain in bed. His arm was swollen and very painful; the erythema extended over the whole arm and down the right side of the chest. The second day was not quite as bad, while on the third he was able to come to the office. His arm was still swollen, the erythema gradually subsiding. The group of doctors who saw this reaction were greatly impressed.

A year later, in 1926, Dr. H. R. M. made it possible for the skin test to be given a comprehensive trial. The following is the history of a patient on whom was tried the second skin test.

The patient, Mr. C., aged thirty-one, an oil field worker, was seen first on June 17, 1926. He complained of indigestion of several months' duration.

Except for a left inguinal hernia, repaired in 1917 and an attack of influenza in 1922, the patient had always been in good health. During the past year he had been smoking and chewing a great deal of tobacco. Eight months before coming for examination he had had an attack of indigestion which lasted about four weeks. The present attack had been practically continuous for four and one-half months. About one hour after a meal he would have distress and fullness in the epigastrium and a dull burning pain which would last three or four hours or until the next meal was taken, when it would be completely relieved only to return in about one hour's time. He also complained of sour eructations of gas and watery fluid accompanying the burning pain. There was no vomiting. About 10 P.M. or later in the evening he would drink a glass of milk, as this would relieve the distress and burning for about an hour. Taking bicarbonate of soda also relieved, but for a shorter time.

Physical examination was negative except for tenderness in the abdomen over region of the pylorus. Urinalysis was negative. Blood count was normal. Fractional gastric analysis. The fasting stomach contained about 100 c.c. of fluid, showing free HCl 62° and total acidity 95°. The digestion curve gave a fairly normal level of acidity, but the secretion was prolonged. Fluoroscopic examination of the stomach and duodenum showed hyperperistalsis with a poor filling duodenal cap which showed a notch on its inferior border and a moth eaten appearance on its right side. At the six-hour examination the stomach was empty. Diagnosis: Duodenal ulcer.

The skin test was positive after five hours, being characterized by a raised wheal the size of a 25-cent piece and surrounded by slight erythema. This reaction lasted for twenty-four hours without any systemic reaction or discomfort. Dr. H. R. M. and Dr. J. R. were willing to volunteer at once and through their willingness I was able to solicit volunteers for my test. Drs. H. R. M. and J. R. were negative. Shortly after this the discovery was made that a positive reaction could be elicited in patients suffering from asthma, arthritis, hay fever, laryngitis, chronic rhinitis, paranasal sinusitis, colitis, etc. Some reactions were severe, others mild. Those with active focal infections had severer reactions, while others varied accordingly. It seems that each particular illness gives a characteristic reaction. For instance, in asthma those who were positive showed signs of a mild aggravation of symptoms, while others had none. A similar condition was observed in arthritis, etc. It all depended upon the susceptibility of the individual.

From those tested, 19 suffered from arthritis, 8 from peptic ulcer (diagnosed as such either clinically or at operation), 4 from asthma, 3 from hay fever, 7 from chronic rhinitis, 3 from eczema, 6 from colitis, and one patient with exophthalmic goiter having severe tonsillitis. All the above were positive, while 11 out of a total of 62 persons tested, were negative.

It may seem strange that so many positive reactions occur, and one might infer that the organism would elicit a positive reaction in individuals not suffering from this infection at all, but being simply sensitive to the protein and possibly reactive to most any foreign substance thus introduced. However, such reactions can hardly enter, because with rare exceptions the readings are made the next day, that is, twenty-four hours later; some of the severest reactions last as long as seventy-two hours. Others do not make their appearance until the next morning, and those persist for a longer time. Any reaction disappearing before three hours have elapsed is considered negative.

The following history of a patient will illustrate that the organism has a specificity and does not produce a reaction even with triple the quantity of the skin-test injection in individuals suffering from diseases producing a similar clinical picture but due to a different infection. Skin tests were made repeatedly on this patient without success. After study of the bacterial flora of his feces there was isolated a bacterial strain towards which he reacted positively. He improved a great deal after treatment with the autogenous vaccine.

Case No. 2196. March 27, 1926, W. M. P., aged forty-six.

Complaints.—Abdominal distress and gas belching. Headache.

History of present illness.—As a young man, the patient had no digestive trouble, except a tendency to constipation, lassitude, etc. Since having influenza seven years ago, he

had had a good deal of stomach and bowel trouble, characterized by spells of abdominal distress, sometimes amounting to a feeling of soreness over the whole abdomen with gas belching, loss of appetite, lassitude, and frontal headache. Attacks lasted usually a day or two and were separated by brief intervals of almost complete relief. They were provoked by slight dietary indiscretion, constipation, worry, or overwork. No constant relation to meals but apples, salads, etc., were aggravating. Three years ago the patient had a spell lasting almost a week with slight jaundice and pain and tenderness over the gall bladder. Operation was performed with removal of gall bladder (strawberry type) and a chronically inflamed appendix. Exploration of the stomach and duodenum was negative. Very little change after operation.

Diagnosis.—Chronic colitis.

I have observed that in cases where there has been no focus of infection, the reaction was invariably negative. In all cases, however, where the focus existed or had been removed in previous years, a positive reaction was obtained.

Many additional cases could be cited but it is deemed advisable to publish them with the necessary clinical data in another paper. In most of the positive cases stock vaccine made from the organism has been administered with results which warrant detailed description.

CONCLUSIONS

1. Further studies revealed that the organism retained its specificity and selective affinity as described previously.

2. Various strains have been isolated either from foci of infection or from the stomach contents from patients suffering from such infections.

3. With 0.01 c.c. of a 1 per cent suspension of the organism given intradermally, positive reactions were obtained in 19 cases of arthritis, 8 cases of peptic ulcer, 4 cases of asthma, 3 cases of hay fever, 7 cases of chronic rhinitis (some of which had sinusitis, antrum infections and arthritis), 3 cases of eczema, 6 cases of colitis, and 1 case with exophthalmic goiter having severe tonsillitis; 11 cases out of the 62 tested were negative.

4. Although the organism when inoculated into the guinea pig has a selective affinity for the gastric mucosa, etc., it seemingly plays a primary rôle in those infective processes in man which are chronic and latent. Apparently the organism is living in antagonistic and also possibly constructive symbiosis, thus producing the various symptoms enumerated above.

NOTE.—I am indebted to Doctor H. Ross Magee, Anaheim, California, for his interest and helpful criticism.

A STUDY OF THE LARGE DIFFUSE-MARGIN PLAQUE OF SEWAGE FILTRATE*

By Occo Goodwin and Janet Caldwell, Dallas, Texas

DURING the isolation of bacteriophage strains from sewage filtrate (S. F.) an unusually large and peculiar area of lysis was sometimes encountered which differed enough from the classic plaque to warrant a special study.

These lytic areas had been observed at different times with B. typhosus, B. dysenteriae, and B. coli. A stock culture of B. typhosus (No. 8) was selected as the organism with which to produce a purified filtrate for study. The filtrate was obtained and purified from these lytic areas by the usual method of plaque picking. One of the clear centers from B. typhosus plates, where characteristic areas were well separated, was rubbed with a sterile platinum wire and shaken out into a young broth culture of B. typhosus. This was filtered after six hours to remove resistant organisms carried in from the plaque margins, and a second supply of young susceptible organisms was added to a concentration of 3 or 4 billion per c.c. After filtering a second time, a second plating and picking was made, and the fifth serial passage following the second picking was produced in large amount and stored in 10 c.c. quantities for all succeeding experiments. All experiments were performed with this same filtrate 5 (F5) on 1.7 per cent agar unless otherwise stated.

The characteristic appearance of these areas and their contrast with typical plaques can be noted in the illustration. On ordinary beef extract agar (1.7 per cent) they are round, 9 to 12 mm. in diameter with perfectly clear sterile centers 5 or 6 mm. in diameter. The outer portion consists of modified culture apparently in the form of minute individual secondary colonies, slightly more opaque and drier than the normal growth but uniform in size, thus giving by reflected light a satiny zone. The outer limit of the area is vague so that the term "diffuse-margin plaque strain" is used by the authors in referring to filtrates obtained from these areas.

The questions which the following experiments attempt to answer are whether this lytic area has the essential characteristics of the bacteriophage as described by d'Herelle; or whether it is some other form of lysis, or thirdly, if its characteristics proved to be constant, whether it could be considered a separate bacteriophage thus casting doubt on d'Herelle's contention that there is but one bacteriophage with plurality of races only.

TECHNIC

Agar slants and Petri dishes are unsatisfactory for studies of this lytic principle, because the areas are too large to occur well separated in large

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numbers as is necessary in studies of size and for counting. Therefore, agar was poured in porcelain pans 9 by 5 inches to a depth of 7 mm. The pans were covered with brown wrapping paper and sterilized by dry heat. The standard concentration of organisms in the diluted filtrates was 250,000,000 per c.c., and the mixtures of bacteriophage and suspension were distributed over the surface of the pan agar by adding measured amounts with a sterile pipette in the form of a cross and then spreading the fluid uniformly with L-shaped glass rods. No especial trouble with contaminations was encountered.

Preliminary experiments indicated that isolated plaques could be obtained when pans were spread from serial dilutions of the filtrates using 10^{-4} to 10^{-6}.

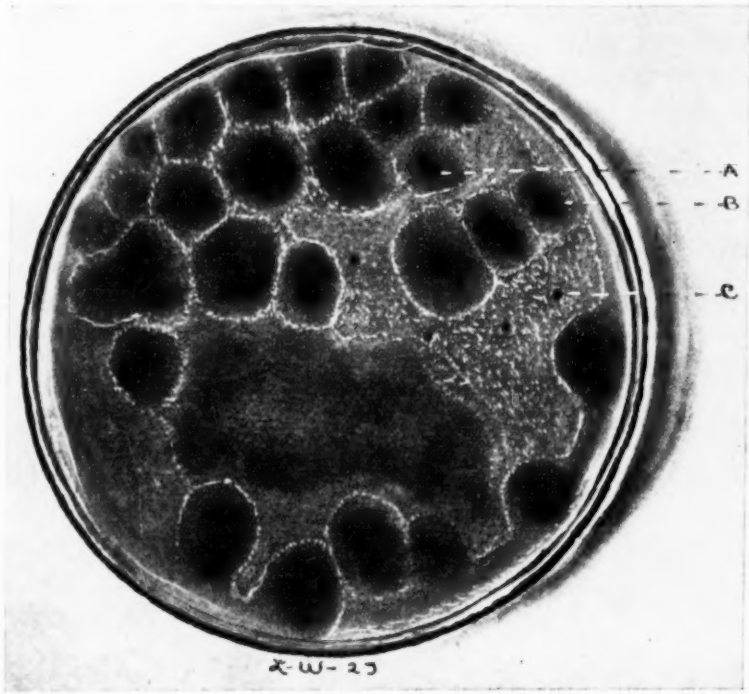

Fig. 1.—A Petri dish culture of B. dysenteriae No. 14 showing many large diffuse plaques and a few 1 mm. sharp-margin placques. *A*, central, sterile area; *B*, outer, satiny zone of changed culture; *C*, 1 mm. plaque.

These dilutions were inoculated with organisms to the standard concentration and 0.5 c.c. was the amount usually spread. When filtrates were produced against other organisms which are susceptible to this lytic substance, they were always allowed to reach a maximum concentration of units by giving them a supply of young susceptible organisms at two different times separated by filtration. This is, of course, a necessary part of the technic with all serial dilution work to insure constant results.

SERIAL LYSIS

The original F5 was passed with the stock B. typhosus (No. 8) through 25 passages. Lysis was observed in each passage, and pans were spread from the filtrate after each fifth serial passage. The plaques were always

typical and they were not changed, apparently, in concentration, as the same dilutions always gave isolated plaques. Thus, it is apparent that the lytic substance of the large diffuse plaque fulfills the two fundamental requirements of the bacteriophage, i.e., lysis in series and isolated areas of lysis on solid media.

SIZE AND APPEARANCE OF PLAQUES

The appearance of the plaques was practically the same on all concentrations of agar with all organisms against which the filtrate showed lytic action, but the size varied both with the organism used and with the concentration of agar. On standard agar (1.7 per cent) the smallest plaques were 9 mm. in diameter with B. paratyphosus B. and B. coli 98U and the largest was 12 mm. in diameter, with several organisms. (See Table I.) With the stock B. typhosus (No. 8) the plaques were almost all the same size but with certain of the other organisms, where the filtrate was not built up against each but only studied in the first or second contact, the plaques varied down to half the usual size. Their characteristic appearance was, however, always retained and the vague margin and satiny zone with the clear center were conspicuous.

The plaques of F5 were observed on agar of concentration from 0.8 per cent to 3.5 per cent, and they were found to vary consistently, being larger the lighter the agar. This is the expected result if these areas behave as do ordinary plaques, for Bronfenbrenner[1] has shown that the latter increase in size with decrease in concentration of agar.

TABLE I

VARIATION IN SIZE OF PLAQUE WITH CONCENTRATION OF AGAR AND TIME OF INCUBATION

ORGANISMS	OUTER DIAMETER (MM.)	INNER DIAMETER (MM.)	PER CENT OF AGAR	INCREASE IN DIAMETER		
				48 HR.	72 HR.	96 HR.
B. typhosus 8 Stock	18	9	0.8			
	16	10	1.0	22 mm.	28 mm.	32 mm.
	16	6	1.2			
	12	6	1.7	15 mm.	24 mm.	
	10	5	2.0			
	8	4	2.5	15 mm.		
	7	4	3.5	11 mm.	18 mm.	
B. typhosus 10 Rawlins	10	3	1.7	15 mm.		
B. paratyphosus B.	9	4	1.7	10 mm.	11 mm.	12 mm.
B. dysentery Shiga	12	6	1.7	17 mm.	18 mm.	
B. typhosus 17	12	6	1.7			
B coli 21U	11	6	1.7	16 mm.	18 mm.	
B. coli 98U	9	4	1.7	12 mm.	15 mm.	

At no time were ordinary sharp-margin plaques seen with these purified filtrates, although dozens of pans and hundreds of plaques were observed during these studies. The fact that the same type of plaque always occurred and alone, indicates that its characteristics are inherent in the lytic substance and are not due to a certain type of resistance in the organisms resulting in unusual secondary colonies. This conclusion is strengthened by the fact that typical sharp-margin plaques have been observed with all these organisms when put in contact with sewage filtrate.

Perhaps the most striking observation made during these studies was the fact that the plaques increased in size after twenty-four hours. The increase was so marked on 1.0 per cent and 1.2 per cent agar that it was noted without measurement and later confirmed by daily measurements. The increase in size was in the outer diameter only as the central, perfectly clear, sterile area remained the same. The outer rim appeared like a slightly depressed line and would often be much more definite twenty-four hours after it could be noted and measured. The culture within this rim became similar to the satiny zone but was never as dry or as conspicuous as the original zone immediately surrounding the clear center. The spread still occurred when the concentration of the suspension was increased to 750,000,000 per c.c. but with 1,000,000,000 or more it was either absent or only slight.

Table I records the increases in diameter from twenty-four to ninety-six hours. As will be seen in the table, the increases occurred with various organisms and on various concentrations of agar, but the increase was greater when the original plaque was large. The figures are the result of several observations with each organism made during other experiments, whenever plaques were well separated and the pans remained free from contaminations. The increases were practically constant. Especially striking were the measurements of B. paratyphosus B. and B. coli 98U whose plaques were always small. In fact it was the small plaque of B. paratyphosus B. found when using a sewage filtrate (No. 5) obtained months after the sewage sample which had been used in obtaining the original large diffuse plaque filtrate that suggested to the authors that this lytic area might be a single entity with a constant type of action on organisms susceptible to it.

As is generally believed in bacteriophagy, plaques do not increase in size nor is there any change in the culture surrounding plaques after the growth is fully developed, say at twenty-four hours, and this characteristic spread of the diffuse plaque forms its chief claim to be considered as a separate bacteriophage species.

To determine whether the increase in size was because the lytic substance spread through the culture or whether it was already present at some distance in the normal appearing culture and produced late changes in it, filtrates were made from strokes through the culture from 2 to 6 mm. from the vague outer border. In every instance lytic action producing similar plaques could be demonstrated in the surrounding culture. Because of drying or late contaminations, these same plaques seldom spread to the point picked but other plaques frequently increased in diameter to this extent. D'Herelle has shown[2] that the bacteriophage penetrates the culture surrounding a plaque over a period of days or even months, but it only succeeds in spreading a few mm. and no macroscopic changes in the adjacent culture are described by him. With the large diffuse plaque the bacteriophage has been recovered in twenty-four hours 15 mm. from the outer margin of the inner clear center, and this is 6 mm. beyond the outer margin of macroscopic change in the culture.

RANGE OF ACTIVITY

The stock F5 was tested with 27 cultures of gram-negative bacilli and was found to have lytic action on only 7. (See Table II.) These cultures include a few stock cultures of B. dysenteriae and B. typhosus but most were isolated in this laboratory from cystoscopic specimens of urine or from blood cultures in cases of typhoid fever. Bacteriophage of marked activity has been found for all these organisms in S.F., so that the limited range of action of F5 was not due to resistance to bacteriophage action on the part of the organisms.

The organisms which were lysed (B. coli 21U and 98U; B. typhosus 8, 10 and 17; B. paratyphosus B. and B. dysenteriae 14) were run again with F5 and pans spread until isolated plaques were obtained to see if only the typical appearances occurred or if difference in the organism produced a change in the plaque. Only the diffuse-margin plaque was ever found, and it was so constant in its appearance that we felt that we must be dealing with a lytic substance of constant and individual characteristics. To test this idea, some of the susceptible organisms were used to isolate from filtrates of various sewage samples, this same type of lytic area. Samples of affluent city sewage had been collected every two or three months during the past year and portions of S.F.2, 3, and 5 were still kept in storage. B. coli 21U and 98U and B. paratyphosus B. were used and purified filtrates produced by the usual process of plaque picking. Most filtrates were purified by 3 or 4 pickings because sharp-margin plaques were also present on the original spreadings but B. paratyphosus B., S.F.5 showed only diffuse-margin plaques from the start and was picked only twice.

Six new purified filtrates were thus produced and these were run against the 27 organisms, and the results are recorded in Table II. In addition to these and the original B. typhosus F5, there was in the senior author's notes a record of a diffuse-margin plaque filtrate isolated six months ago with B. dysenteriae 14. This record though not complete agreed with the others and is included. For contrast the findings of a filtrate purified from a 3 mm. sharp-margin plaque with stock B. typhosus (No. 8) are included at the end of the table.

The technic of this experiment is as follows: A broth culture was made from a fresh agar slant of each organism and incubated eighteen hours. Two filtrates were tested at a time using 5 to 30 drops of each in two broth tubes, respectively, with a control tube. Thus, the racks were set up in sets of 5 broth tubes. The filtrates were pipetted in first and then 3 drops of the broth culture added. Two sterile pipettes were necessary for each organism—one for the first filtrate tubes, the other serving to inoculate the control tube and the second filtrate tubes. Observations were made after three, seven, and twenty-four hours incubation. Agar slants were spread from all 30-drop tubes between the sixth and seventh hours.

There was not one discrepancy in the entire experiment. Every filtrate lysed the same few organisms and was completely negative with the rest. The agreement was perfect even in degree. For instance B. typhosus No. 8 was lysed by every filtrate to a maximum degree at three and seven hours

TABLE II

DEMONSTRATES IDENTITY OF THE LARGE DIFFUSE PLAQUE PURIFIED FROM VARIOUS SEWAGE SAMPLES BY MEANS OF A VARIETY OF ORGANISMS

ORGANISMS	B. T. F. 5 ORIGINAL	B. PARA B. S. F. 2	B. PARA B. S. F. 3	B. PARA B. S. F. 5	B. COLI 98U S. F. 5	B. COLI 21U S. F. 3	B. COLI 21U S. F. 5	B. DYS. 14 ORIGINAL	B. T. SMALL PLAQUE F.
B. coli 2	–	–	–	–	–	–	–	–	+
B. coli 13U	–	–	–	–	–	–	–	–	–
B. coli 21U	+	+	+	+	+	+	+	+	+
B. coli 43U	–	–	–	–	–	–	–	–	–
B. coli 49U	–	–	–	–	–	–	–	–	–
B. coli 98U	+	+	+	+	+	+	+	+	–
B. aerogenes 27U	–	–	–	–	–	–	–		–
B. aerogenes 30	–	–	–	–	–	–	–		–
B. pyocyaneus 3	–	–	–	–	–	–	–		–
B. typhosus 1	–	–	–	–	–	–	–		–
B. typhosus 2	–	–	–	–	–	–	–		–
B. typhosus 4	–	–	–	–	–	–	–		–
B. typhosus 5	–	–	–	–	–	–	–		+
B. typhosus 6	–	–	–	–	–	–	–		–
B. typhosus 7	–	–	–	–	–	–	–		+
B. typhosus 8	+	+	+	+	+	+	+	+	+
B. typhosus 9	–	–	–	–	–	–	–		+
B. typhosus 10	+	+	+	+	+	+	+		+
B. typhosus 17	+	+	+	+	+	+	+		+
B. typhosus 20	–	–	–	–	–	–	–		–
B. typhosus 22	–	–	–	–	–	–	–		–
B. paratyphosus A	–	–	–	–	–	–	–		+
B. paratyphosus B	+	+	+	+	+	+	+		+
B. dysentery 14 Shiga	+	+	+	+	+	+	+	+	+
B. dysentery 15 Shiga	–	–	–	–	–	–	–		+
B. dysentery 16 Flexner	–	–	–	–	–	–	–		–

and showed moderate lysis at twenty-four hours. Twenty-one U, however, though perfectly clear through seven hours, showed no lysis at twenty-four hours. None of the other organisms showed more than weak lysis after the seven-hour reading. Apparently early secondary cultures are the rule with this bacteriophage, and despite the large size of the plaques and the constancy with which sterile agar slants are obtained, it is obviously inferior to sharp-margin plaque strains where permanent marked lysis can usually be obtained with this same group of organisms. Even after the 25 passages with B. typhosus no improvement in the degree of clearing at twenty-four hours occurred so that its virulence is not built up rapidly as are most bacteriophage strains obtained from S.F. This plaque is an exception to the rule that large plaques mean a virulent bacteriophage, for although the plaque is large with a variety of organisms, with none of them is a really satisfactory permanent lysis obtained. The same type of agar slants occurred; usually they were practically sterile with only scattered colonies varying in size. Ragged fringes do not occur on slants with this bacteriophage, as the plaques are so large that when fused the small area of a slant is sterilized, and only the few resistant organisms form scattered colonies over its surface.

We have at present no explanation to offer for the peculiarities of the lytic substance described, but investigation of it has by no means been exhausted. It was at first supposed that it might be a larger particle or be carried by larger particles than ordinary bacteriophage units, but it passed readily through collodion sacs of the following composition:

Parlodion—2 per cent in equal parts of ether and 95 per cent alcohol.

Parlodion—3.5 per cent in 2 parts of ether and 1 part 95 per cent alcohol.

Still less permeable membranes should be tried to see whether or not a membrane could be found which would let ordinary plaque-producing strains through but retain this substance. This possibility was suggested by the work of Bronfenbrenner[3] who has succeeded in fractionating different sized plaque races of bacteriophage by ultrafiltration.

DISCUSSION

As has already been stated the lytic substance just described has two of the fundamental characteristics of the lytic principle described by d'Herelle. It produces lysis in series and in isolated areas, plaques. Thus the substance must be admitted as belonging to the class of phenomena called bacteriophage.

It differs in that it is much larger than any plaque noted by d'Herelle or hitherto described and it has a characteristic outer zone of changed culture which spreads to enlarge the outer diameter over a period of days. The other distinctive feature of the substance brought out in this paper is that it is apparently an entity whose characteristic size, appearance, and range of activity are always the same, no matter from what sewage sample it is fished nor with what susceptible organism it is isolated.

Whether it is only one of the races of the bacteriophage or a separate bacteriophage species, perhaps cannot be answered by these studies, but the variation from type of this substance is different from the variations claimed

by others as bases for forming separate bacteriophage species. In his latest book d'Herelle[4] summarizes these claims. These, briefly, include difference of various bacteriophage strains in antigenic properties; difference in resistance to various harmful reagents; the well-known fact that acquired resistance to one bacteriophage may be accompanied by sensitiveness to a strain previously ineffective on the culture, and, finally, difference in form of lytic colony. The latter contention up to the present has meant only difference in size of plaques in which few would dispute d'Herelle that it could scarcely be the basis for separate bacteriophage species, depending as it does on so many variables such as virulence, nature of growth of organism, concentration of agar.

D'Herelle does not accept any of these claims as sufficient for establishing the plurality of the bacteriophage and answers well the arguments advanced by their proponents. None of these claims involve variations in fundamental characteristics of the bacteriophage which could not be accounted for by adaptation unless, possibly, it is the variation in antigenic properties. As the suitable separation of the bacteriophage from bacterial proteins has only been accomplished recently, it is probable that these variations were due to traces of bacterial proteins of distinct antigenic quality in the filtrates. At least such work will have to be confirmed with bacteriophage solutions purified according to recent careful methods.[5]

The substance here described does differ, however, in one of the accepted essentials of bacteriophage phenomena—the permanency of the plaque in size. D'Herelle and others lay much stress on this permanency and also consider the sharp outer margin as a constant feature. When we consider the striking peculiarities in appearance of the diffuse-margin plaque and couple this with the proved singleness of the substance, we believe that it has a greater claim as a separate bacteriophage species than any previously advanced claim.

SUMMARY

A plaque has been described which averages 12 mm. in diameter and which has an outer zone of changed culture that increases gradually in size to enlarge the diameter of the plaque as much as 100 per cent.

The plaque has been isolated from several samples of sewage collected during a nine-month period and all these plaques have identical characteristics and their filtrates have the same range of activity in a group of twenty-seven bacilli.

The peculiarities and singleness of the substance are believed to be sufficient basis for considering it as a separate bacteriophage species.

NOTE: Hadley has described similar lytic areas in a culture of paratyphoid bacilli (Proc. Soc. Exp. Biol. and Med., February, 1928, xxv, 359) and finds that ordinary plaques do occur in pure line cultures of the large plaques. He considers the two types to be different components of the bacteriophage.

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BARBITAL IN PARATHYROID TETANY*

By L. B. Brannon, M.S., and C. A. Dragstedt, Ph.D., M.D., Chicago, Ill.

THE convulsive phenomena seen in parathyroidectomized animals and attributed to hyperirritability of the peripheral nerves, spinal cord, midbrain, and cerebellum are probably all due to an alteration in the chemistry of the blood and lymph. At present the preponderating evidence would indicate that this alteration involves primarily the blood calcium, the concentration of which is generally reduced. Thus, various procedures which increase the concentration of calcium in the blood will mitigate the parathyroprival symptoms.

Nevertheless, there are a number of procedures which suffice to control or relieve tetany without materially affecting the calcium level of the blood. Thus Dragstedt,[1, 2] using strontium salts, was able to keep parathyroidectomized animals alive for twelve days, although he concluded that strontium was not as effective as calcium. The results with magnesium chloride are even more striking. Luckhardt, et al,[3] and later Wenner,[4] showed that parathyroidectomized dogs could be kept alive with magnesium salts administered orally. This is surprising in the light of the well-known fact that magnesium given parenterally causes an increase in calcium elimination, whereas even parenterally-administered magnesium sulphate is now known to have a favorable effect on parathyroidectomized animals.[5] This strongly suggests that magnesium, though it might act (as strontium probably acts) by virtue of its chemical relationship to calcium so as to restore some hypothetic ionic equilibria, may instead act by virtue of a general depressing effect upon the nervous system, so that it would tend to prevent the onset of violent or fatal convulsions. This would be in harmony with the observation of Sloan[6] that parathyroidectomized dogs could be carried through attacks of tetany, with considerable prolongation of their lives, by the use of morphine sulphate.

Inasmuch as Tatum, et al,[7] have shown barbital to be very effective in controlling convulsions, we were led to study the effect of barbital in controlling the tetany of parathyroidectomized dogs.

Methods.—Twelve dogs were used in this study. They were etherized and the extirpation of thyroids and parathyroids was done by the usual technic. After recovery from the anesthetic they were fed daily one-half pound of meat and one-half pound of bread; this was given by stomach tube when the dog refused to eat. Sodium barbital was administered either orally or subcutaneously in doses of one gram. This was given daily or as the presence of tetany indicated. Blood-calcium determinations were made according to the Kramer-Tisdall method.

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Results.—Tetany developed in one dog in twenty-four hours; in eight animals in forty-eight hours; in two animals in seventy-two hours, and in one animal in ninety-six hours. The longest period of survival was fourteen days. The shortest survival was in an animal which, dying on the fifth day, was found to have a bilateral bronchopneumonia. The average survival period for the series was nine days. This is considerably longer than the average survival period of untreated dogs when kept on a meat diet. Following the administration of the barbital, the tetany subsided slowly as the animal became depressed and did not reappear until the barbital effect had worn off—usually from thirty-six to forty-eight hours. In only two animals did tetany reappear within twenty-four hours. In no instance was there a rise in the blood calcium coincident with the relief from tetany, the animals being free from tetany with the calcium level varying from 5.5 to 6.8 mg. per 100 c.c.

All dogs died in depression, five of them showing bronchopneumonia at autopsy. This infection, we believe, brought down the average survival period quite markedly, as the survival periods for these animals were 6, 5, 6, 4 and 8 days, while for the others they were 14, 11, 11, 7, 11, 12 and 10 days.

The following is a typical protocol:

Dog 39—male, 12 kilo.
July 14, 1927—Blood calcium 10.7. Thyroparathyroidectomy.
July 15, No tetany.
July 16, Tetany, barbital 1.5 gm. Tetany relieved in 4 hours. Blood calcium 7.2.
July 17, Tetany, barbital 1 gm. Tetany relieved in 4 hours.
July 18, No tetany.
July 19, No tetany, barbital 1 gm.
July 20, Slight tetany. Blood calcium 6.5.
July 21, Tetany, barbital 1 gm. Tetany relieved in 4 hours.
July 22, No tetany.
July 23, Slight tetany, barbital 1 gm. Tetany relieved in 4 hours. Blood calcium 5.4.
July 24, No tetany. Depression.
July 25, Dead.

DISCUSSION

It appears from this study that tetany may be controlled and the life of a completely thyroparathyroidectomized animal prolonged by a depressant, such as barbital, without any material alteration in the blood calcium, even though the animals are kept on a meat diet. Whatever the cause of tetany, the tetany itself presumably does some damage to the central nervous system which hastens the fatal issue.

CONCLUSIONS

Parathyroid tetany may be relieved or controlled by the adequate administration of barbital without any material change in the level of the blood calcium. The life of the parathyroidectomized dog can be slightly prolonged when tetany is controlled in this way.

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INTERMITTENT PARTIAL HEART-BLOCK*

Report of a Case

By William D. Reid, M.D., Boston, Mass.

CASE History: Physician, aged forty-one years, seen January 28, 1926. He had recently had an electrocardiogram taken as a matter of interest, not because of being ill. This electrocardiogram disclosed a P-R interval of 0.34 second; because of this unexpected finding the patient desired further study.

Past history: Patient has had hay fever and asthma since as early as he can remember. This was found to be due to ragweed pollen, and he has been relieved by prophylactic injections. He underwent tonsillotomy at the age of five, and adenoidectomy when nine years old. He states that he nearly died after the latter operation, and believes that it was due to shock. He was kept out of school between the ages of thirteen and fourteen because of probable pulmonary tuberculosis; he made a good recovery. Following a throat operation at the age of nineteen, he experienced a severe hemorrhage. He was interested in athletics while in college.

The patient denies any form of rheumatism, chorea, or evidence that his heart was affected. Wassermann was negative; he denies venereal infection.

Physical examination: The patient was tall, and well developed. Heart impulse was barely palpable in fifth interspace at nipple line. Percussion borders within normal limits. Action regular, no murmurs detected. Heart rate 63, and 60 after exercise (sitting up and lying down slowly six times). Blood pressure: systolic 118; diastolic 85.

Patient has been seen repeatedly since January, 1926 and has shown nothing new of significance. In August, 1926 he climbed Mt. Royal near Montreal, Canada, without undue fatigue. A little later he swam one mile in salt water with apparent ease. Remainder of history and examination contains nothing of interest.

ELECTROCARDIOGRAMS

The variation in the duration of the P-R interval is the chief item of interest. This may be followed in Table I. In the third, ninth, and twelfth record the duration of the P-R diminished to normal or nearly so. The shortening in the electrocardiogram taken by Dr. R. W. Scott was apparently spontaneous; the patient could give no cause other than that he was on vacation and feeling very well. The accompanying illustrations have been selected as the more interesting of these records.

Pressure on the right or left vagus nerve in the neck produced an equal degree of lengthening in the conduction time. The electrocardiograms

*From the Evans Memorial.

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taken September 27, 1927, are of considerable interest in that the P-R interval shortened to a high normal figure immediately after strenuous exercise, and is shown to be lengthening progressively to its previous abnormal figure one minute subsequently. The cardiac rate is accelerated in the records immediately after exercise and after atropine, but in the one taken by Dr. Scott the P-R is relatively short, though the heart rate is about as in the majority with prolonged conduction times. The duration of the P-R interval does not, therefore, closely follow the heart rate.

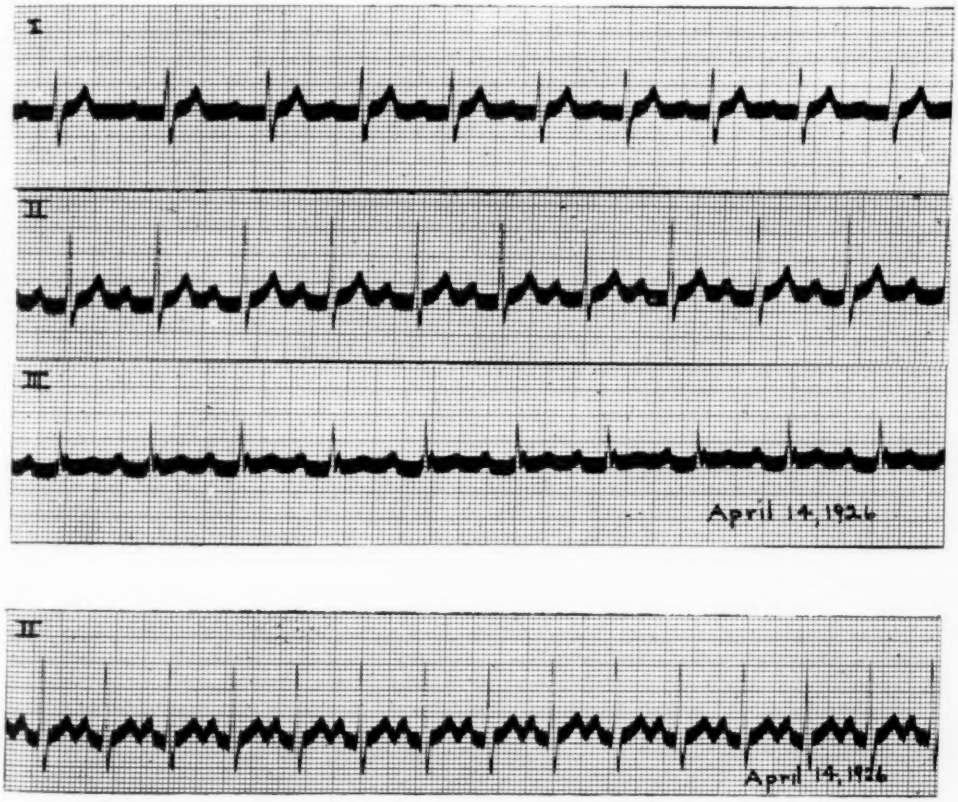

Fig. 1.—Above: Electrocardiogram under normal conditions. P-R = 0.28 sec. Heart rate = 80-89. Below: Lead II twenty-five minutes after injection of atropine sulphate, gr. 1/30. P-R = 0.18 sec. Heart rate = 119-121.

COMMENT

As far as I am aware such variation in the duration of the auriculoventricular conduction (P-R interval) is unusual. There is comparatively little literature dealing with such a condition.

Wiggers[1] states: "Heart-block of a transient nature may be due to excessive vagus influence and, in predisposed cases, may indeed be induced by vagus compression . . . ." Gager[2] reported a case of partial heart-block occurring during the height of a pericardial effusion and disappearing on withdrawal of the fluid by paracentesis of the pericardium. He believed that in this case the delayed conduction was caused by an impaired blood supply

TABLE I

DATE	P-R (SECONDS)	HEART RATE	REMARKS
January, 1926	0.34		Taken in another laboratory.
April 14, 1926	0.28	80-89	
April 14, 1926	0.18	119-121	30 minutes after 1/30 gr. atropine.
April 20, 1926	0.26	62	
April 20, 1926	0.26 0.32	58-62	Pressure on left vagus.
April 20, 1926	0.26 0.32	58 65	Pressure on right vagus.
June 15, 1926	0.24-0.28	58-63	
June 29, 1926	0.24-0.28	58 61	
August 8, 1926	0.21-0.22	71-78	Taken in Cleveland, Ohio, by Dr. R. W. Scott.
September 9, 1926	0.22-0.24	72-74	
June 15, 1927	0.24	70	
September 27, 1927	0.24	73-83	
September 27, 1927	0.20	104-109	After one minute of strenuous exercise.
September 27, 1927	0.21 0.24	83 86	One minute later.

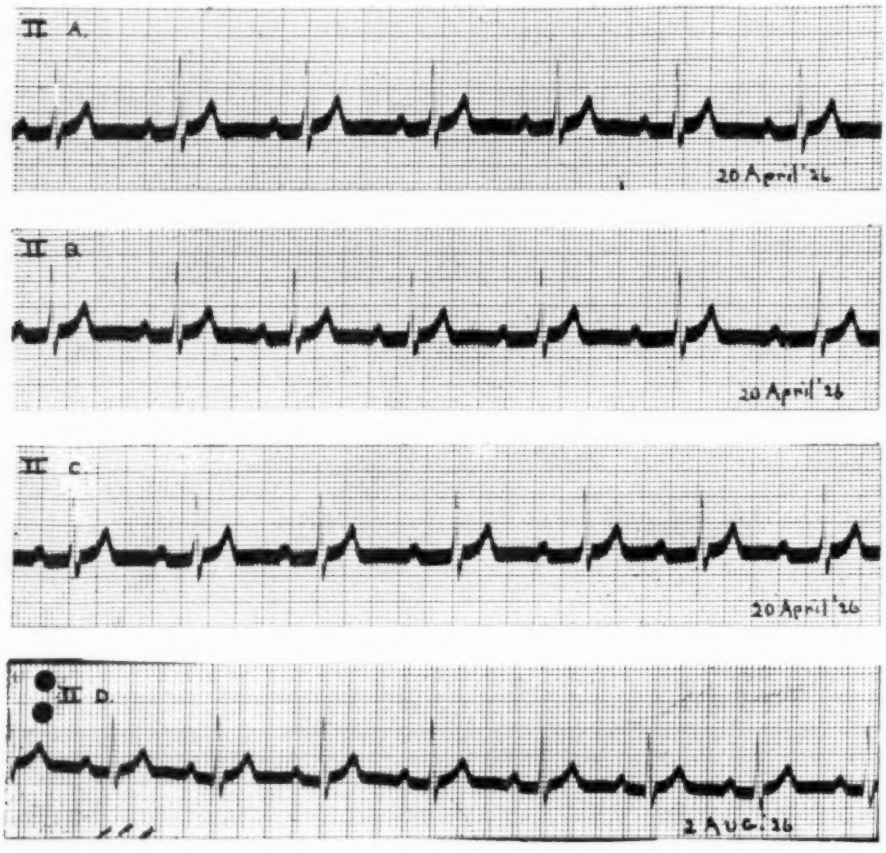

Fig. 2.—Four records of Lead II, dates on prints. *A*, Normal conditions: P-R = 0.26 sec. Heart rate = 62. *A*, During pressure on left vagus. P-R = 0.26-0.32 sec. Rate = 58-62. *C*, During pressure on right vagus. P-R = 0.26-0.32 sec. Heart rate = 58-65. *D*, Normal conditions. P-R = 0.21-0.22 sec. Heart rate = 71-78. (Courtesy of Dr. R. W. Scott.)

to the bundle of His, as a result of direct interference with the intrinsic cardiac circulation by the pressure of the effusion on the coronary arteries and veins.

Gager refers to a few other reports concerning changes in the blood supply causing partial heart-block, and believes such changes may explain some cases of transient or even more definite heart-block in which at autopsy the bundle of His may be found to be essentially normal.

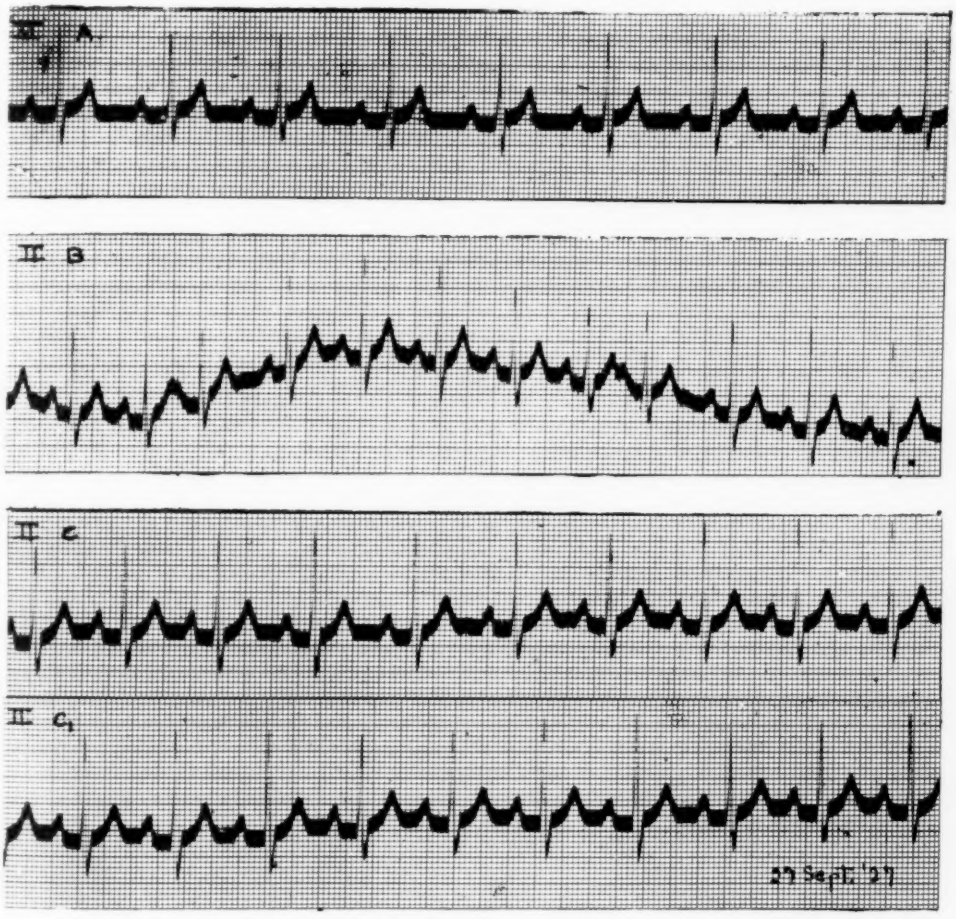

Fig. 3.—Three records of Lead II, all taken Sept. 27, 1927. *A*, Normal conditions: P-R = 0.24 sec. Heart rate = 73-38. *B*, Immediately after exercise: P-R = 0.20 sec. Heart rate = 104-109. (Rise in the base line of the string shadow is due to shifting of the position of the heart during the postexercise dyspnea.) *C and C*$_1$. Directly continuous record taken one minute after *B*. The P-R shows a gradual lengthening from 0.21 to 0.24 sec. Heart rate = 83-86.

Maron and Winterberg[3] describe a case of rheumatic heart disease with mitral stenosis in which partial heart-block with marked variation in the duration of the P-R interval was recorded. They found the following variation:

P-R	= 0.46	second,	pulse	rate	75,	Dec. 8,	1920
"	= 0.40	"	"	"	62,	" 10,	"
"	= 0.25	"	"	"	69,	" 12,	"
"	= 0.21	"	"	"	66,	" 20,	"
"	= 0.44	"	"	"	71,	" 30,	"

Immediately after exercise this P-R shortened to 0.18-0.19 second, but increased after the next few beats to 0.50 second, and then varied between 0.27

and 0.28 second. This rapid return to a prolonged P-R is similar to the case I am reporting.

A case somewhat more similar to my case is that of a medical student[4] devoted to out-of-door sports, especially skiing. He had never noted any signs of heart disturbance during strict physical training and nothing abnormal was found on clinical or x-ray examination, yet electrocardiograms disclosed a P-R interval of almost twice the normal duration. Under the effect of exercise and atropine, the heart-block disappeared entirely.

In the case I am reporting, it is not clear why such variation in the P-R interval occurs. Its reduction to 0.18 second, which is within the limits of the normal duration, does not, as is well established, prove that the delay is necessarily solely dependent upon the influence of the vagus; the latter does not usually produce a prolongation to the degree or as persistent as that found in my patient. The bundle of His may not be normal, and yet under ordinary conditions may still conduct within the normal time limits. In such a case, however, the P-R interval may be lengthened to an abnormal period by the influence of the vagus upon a bundle of His which is already imperfect in function. It is conceivable that in this case the conducting bundle is congenitally imperfect or may have received some injury from an unrecognized infection; the variations in the duration of the P-R interval may then be due to variations in the vagus influence.

The observation of this patient serves to modify somewhat the rather serious interpretation usually applied to the finding of partial heart-block.

SUMMARY

A case of partial heart-block with considerable variation in the length of the P-R interval, and occasional reduction to a normal duration spontaneously, after atropine, and immediately after exercise, is reported. The history and physical examination give no evidence of abnormality; the patient apparently is in good health.

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MORPHINE TOLERANCE*

I. The Acquirement, Existence and Loss of Tolerance in Dogs

By Ardrey W. Downs and Nathan B. Eddy, Edmonton, Alberta, Canada

In preparation for experiments reported elsewhere[1] upon the susceptibility of morphine tolerant animals to allied drugs and to cocaine, we habituated six dogs to large doses of morphine. In each case the initial dose was 50 mg. of the sulphate per kilogram of body weight. This was increased as the condition of the animal warranted until a dose of 150 mg. per kilogram was reached. The salt was dissolved in 0.9 per cent sodium chloride solution, 50 mg. per 1 c.c., and administered subcutaneously. The injections were made daily except Sunday. The usual increase in the dose was 10 mg. per kilogram, and two weeks were required to work up to the maximal dose which was continued for three weeks. One of the dogs early showed marked resistance to the drug, and in her case the maximum dose was increased to 200 mg. per kilo. One dog died on the twenty-first day of the experiment and another on the twenty-fifth day. The dog that died first had received the maximal dose four times and the other nine times. The cause of death could not be determined in either case.

Table I

Initial Dose

Dog No.	Behavior		Pupil		Pulse		Respiration		Temperature		
	Before	After	Before mm.	After mm.	Before	After	Before	After	Before ° C.	After ° C.	
15	Active	As'eep	8	5	130	108	150	40	38.8	38.5	Defecated
20	Active	Drowsy	6	6	120	50	42	96	39.7	39.2	Vomited
42	Quiet	Asleep	6	5	96	64	22	46	39.6	39.5	
45	Restless	Quiet	7	8	138	96	43	180	39.5	39.0	Vomited
93	Restless	Asleep	7	6	150	48	188	64	40.0	40.0	
99	Active	Drowsy	9	7	120	96	18	160	39.0	39.2	Vomited
Average			7.1	6.1	126	77	77	98	39.4	39.2	

We adopted the degree of narcotic effect as the index of the action of the drug. In addition the width of the pupil, pulse rate, respiratory rate, and temperature were recorded before the administration of the morphine and thirty minutes after. These observations on each dog before and after the injection of the initial dose are set forth in Table I, before and after the first maximal dose in Table II, and before and after the last dose in Table III. Comparison of these tables shows adaptation of the mechanisms controlling vital activities to the action of the drug. The effects of a dose of morphine after the dogs had become accustomed to a fairly large dose were similar in kind to those produced by the initial dose, though usually less in degree.

*From the Department of Physiology and Pharmacology, University of Alberta.
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TABLE II

FIRST MAXIMAL DOSE

DOG NO.	BEHAVIOR		PUPIL		PULSE		RESPIRATION		TEMPERATURE		
	BEFORE	AFTER	BEFORE MM.	AFTER MM.	BEFORE	AFTER	BEFORE	AFTER	BEFORE ° C.	AFTER ° C.	
15	Normal	Slightly drowsy	8	6	100	132	12	15	38.7	38.0	
20	Quiet	Quiet	8	8	76	96	15	18	38.9	38.2	
42	Quiet	Walking about	6	4	72	76	16	20	38.7	38.5	Defecated
45	Quiet	Quiet	7	7	100	104	27	26	39.4	39.4	
93	Normal	Restless	7	7	124	120	18	28	39.5	39.2	Defecated
99	Slightly depressed	Slightly depressed	9	9	88	116	12	16	38.6	38.8	Convulsion
Average			7.5	6.8	93	107	17	20	38.9	38.6	

TABLE III

LAST DOSE

DOG NO.	BEHAVIOR		PUPIL		PULSE		RESPIRATION		TEMPERATURE	
	BEFORE	AFTER	BEFORE MM.	AFTER MM.	BEFORE	AFTER	BEFORE	AFTER	BEFORE ° C.	AFTER ° C.
15	Quiet	No depression	7	5	99	76	18	16	39.0	38.6
20	Quiet	Quiet	9	8	81	110	21	30	39.0	38.8
42	Quiet	No depression	4	4	118	86	20	16	39.1	38.6
45	As usual	No depression	8	7	122	110	38	30	39.5	38.9
93	As usual	No depression	7	10	160	138	10	26	39.8	39.8
99	As usual	No depression	9	8	92	136	25	23	38.9	38.7
Average			7.3	7	112	108	22	23	39.2	38.9

The initial dose produced depression of psychical activity. If roused and made to move about, each dog showed marked weakness and incoordination in the hind limbs. This was not noticed in any animal after the third injection. As the treatment continued, all the dogs became quieter but not particularly more drowsy after the injection than before. Dogs 15 and 45 showed a decided change in character which was especially marked in Dog 45. Her manner became slinking and furtive in contrast to the free and rather disdainful fashion in which she had trotted about the room at first. Dogs 15, 42, and 45 quite evidently had hallucinations. We were never able to detect any desire for the injection on the part of any of the dogs. Nevertheless the effect of abstinence from the drug for even one day was pronounced. On Monday morning the dogs were always much more active than usual.

Sollmann[2] states that in the dog the effect of morphine on the pupil is variable but generally dilator. After the first dose of morphine we found the pupil to be constricted in four of the six dogs, unchanged in one, and slightly dilated in one. The average reduction in the size of the pupil was 14 per cent. The last dose of morphine also constricted the pupil slightly in four of the six dogs, left it unchanged in one, and dilated it in one. The average reduction in the diameter now was only 4 per cent.

The first dose of morphine decreased the pulse rate markedly, 38.88 per cent. Later the heart rate averaged less before the giving of the morphine than at the beginning of the experiment. At the time of administration of the first maximal dose the decrease was 26 per cent. This might have been due to persistent effect of the morphine or it might have been the result simply of the quieter behavior of the animals at this time. However, thirty minutes after the injection of the maximal dose of morphine the pulse rate increased in all but one animal. The average result was an increase of 15 per cent.

Van Egmond[3] claims that in the habituation of dogs to very large doses of morphine the vagus center retains its responsiveness to morphine almost unchanged.

Recently McRea and Meek[4] have reported experiments tending to show that the slowing of the heart by morphine is not more than partly due to direct stimulation of the vagus center, but that it is rather principally secondary to the depressant effect of morphine on the brain. If we accept their view, it may be that the vagus center does fail to become completely tolerant to morphine and that absence of the slowing effect upon the pulse in the habituated animal is due to absence of the depressant effect on the brain.

Heinekamp[5] has observed that adrenalin produces a greater degree of slowing of the heart after the previous injection of a small dose of morphine. He attributes this to synergism between morphine and adrenalin on the vagus center. Persistence of this synergistic action in an animal habituated to morphine should indicate lack of tolerance on the part of the vagus center. Accordingly, we administered adrenalin to three tolerant dogs and to three control dogs. Each received an intramuscular injection of 0.1 c.c. per kilogram of body weight of adrenalin chloride solution, 1:1000. The pulse rate was noted before the injection and for the ten minutes immediately follow-

TABLE IV

EFFECT OF ADRENALIN ON THE RATE OF THE HEART

DOG NO.	NORMAL DOGS				DOG NO.	HABITUATED DOGS			
	BEFORE MORPHINE		AFTER MORPHINE			BEFORE MORPHINE		AFTER MORPHINE	
	BEFORE ADRENALIN	AFTER ADRENALIN	BEFORE ADRENALIN	AFTER ADRENALIN		BEFORE ADRENALIN	AFTER ADRENALIN	BEFORE ADRENALIN	AFTER ADRENALIN
1	156	122.7	108	97.3	15	128	117.9	144	128.4
2	120	136.1	120	103.9	20	81	88.0	110	91.0
3	96	93.8	72	48.7	93	160	153.0	138	143.5
Average	124	117.5	100	83.3	Average	123	119.6	130.6	120.9
Change in per cent		-5.24		-16.7	Change in per cent		-2.76		-7.42

ing. The habituated dogs then received their usual dose of morphine and a second dose of adrenalin thirty minutes later. The pulse rate was counted as before. The dose of morphine sulphate given the control animals was 10 mg. per kilogram. This was followed by the same dose of adrenalin as given previously. The counting of the pulse rate was carried out as described. The average pulse rates per minute are given in Table IV. From these results it would seem that the vagus center is still responsive in the habituated dogs to the stimulating action of adrenalin and of the increased blood pressure due to adrenalin, and also that after morphine the vagus center in both normal and habituated dogs is more sensitive than before.

On the following day the dogs were much more depressed than usual and two, Nos. 20 and 93, died. The death of these dogs was mentioned early in this report, and the statement made that the cause of death could not be determined. Two possibilities present themselves. The adrenalin may have broken their tolerance to morphine so that the dose of 150 mg. of morphine sulphate per kilogram of body weight was more than they could stand, or the adrenalin may have sensitized the nerve centers so that 150 mg. became equivalent to some much larger dose. Dog 15 was aroused with difficulty the next morning and no morphine was given. On the following day 50 mg. caused very great depression, but during the next four days it was possible to return gradually to the usual dose of 150 mg. per kilogram.

Two weeks after morphine had been discontinued, the three survivors among the habituated dogs, Nos. 15, 42, and 99, and two of the control dogs, Nos. 1 and 2, were given adrenalin chloride solution of the same strength and in the same dose as before. The dose was repeated after forty-five minutes, but the morphine was omitted. No depression was observed in any animal. Still later, five weeks after the withdrawal of morphine, the same dogs were given a single similar dose of adrenalin chloride solution followed immediately by morphine. The dose of morphine sulphate for the control dogs was 10 mg. per kilogram of body weight and for the others 50 mg. per kilogram. The results were compared with those of a single similar dose of morphine in the control dogs and of the initial dose in the others. We were unable to detect any greater depression than with morphine alone.

There was one effect of the intramuscular injection of adrenalin which should be noted. In every case there was a local reaction—the leg became swollen and painful and in most instances a discharge of necrotic tissue began on the seventh or eighth day. Probably the necrosis was due to constriction of the arterioles at the site of injection. Biberfeld[6] reports the subcutaneous injection of protein (milk) as effecting a temporary break in the tolerance of dogs to successive doses of morphine. This leads us to wonder whether the great depression produced by morphine and adrenalin in the habituated dogs might have been due to a break in tolerance caused by necrosis and the entrance of protein degradation products into the circulation.

Morphine always reduced the body temperature. The reduction was about the same whether we consider the effect of the first or of the last dose.

Vomiting occurred in three of our dogs after the first dose of morphine only. Later, about the time the maximal dose was reached or shortly before,

all the dogs became salivated. This condition persisted until after the withdrawal of the morphine. This might indicate tolerance of the vomiting center to the depressant effect of morphine. Apomorphine was employed to test the possible existence of such tolerance.

Three normal dogs were selected and given apomorphine subcutaneously. The apomorphine was dissolved in normal saline to make a 0.1 per cent solution. Two of the dogs received 0.2 mg. apomorphine per kilogram of body weight and one received 0.4 mg. per kilogram. All vomited. The average time until the first vomiting was six minutes, fifty-seven seconds, and the average number of times a dog vomited was twenty-five. Five days later the same dogs were given morphine sulphate, 10 mg. per kilogram, and forty-five minutes afterward 0.2 mg. apomorphine per kilogram. None vomited after the apomorphine.

The dogs that had been habituated to morphine were given their morphine and thirty minutes later 0.2 mg. of apomorphine per kilogram of body weight. All vomited. The first vomiting occurred at an average interval of eight minutes, thirty-one seconds, after the apomorphine and the average number of times each vomited was five. A comparison of these results in normal dogs without and with morphine and in dogs that had been receiving morphine regularly justifies the conclusion that the dogs of the latter group were tolerant to morphine in the amounts administered.

All of the dogs lost weight rapidly during the first week. This was principally because they were so depressed that they ate little. However, from the second week on their weight remained stationary or showed a slight gain.

TABLE V

CONDITION OF SURVIVING DOGS TWO WEEKS AFTER LAST DOSE OF MORPHINE

DOG NO.	BEHAVIOR	PUPIL MM.	PULSE	RESPIRATION	TEMPERATURE ° C.
15	Active	5	148	24	38.9
42	Active	4	136	28	39.0
99	Active	5	152	30	39.1
Average		4.7	145	27	39.0

When the morphine was withdrawn, no attempt was made to reduce gradually but there was simply a sudden stoppage of the daily dose. Previous to this Dog 45 had been killed by cocaine. The three survivors showed no ill effects of this drastic procedure but instead manifested an increasing liveliness, so that at the end of two weeks they were all restored to their original condition in this respect (Table V). Salivation persisted five days after cessation of the morphine in one and eleven days in each of the others.

SUMMARY

Dogs habituated to the daily subcutaneous injection of morphine sulphate, 150 mg. per kilogram, showed a high degree of tolerance to the depressant action of the drug on the cerebrum and on the vomiting center. Tolerance to the action of morphine on the heart rate and on the size of the pupil was also shown. Body temperature was always lowered by morphine.

We are indebted to Mr. J. P. Quigley for assistance in making the observations recorded in this report.

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MORPHINE TOLERANCE*

II. The Susceptibility of Morphine Tolerant Dogs to Codeine, Heroine and Scopolamine

By Ardrey W. Downs and Nathan B. Eddy, Edmonton, Alberta, Canada

DOGS which had been rendered tolerant to morphine as described previously[1] were subjected to the action of codeine, heroine and scopolamine in the hope that it might be possible to arrive at some conclusion in regard to the existence of cross tolerance.

Codeine.—Two normal dogs were first given codeine and subsequently three morphine habituated dogs were given codeine. The dose for one of the control animals was 20 mg. of codeine phosphate per kilogram of body weight and for the other 40 mg. Two of the tolerant dogs had been receiving 150 mg. of morphine sulphate per kilogram of body weight daily. To one of them 40 mg. of codeine phosphate per kilogram was given, and to the other 80 mg. The third morphine habituated dog was accustomed to 200 mg. of morphine sulphate per kilo and she was given 100 mg. of codeine phosphate per kilogram. The drug was made up in 5 per cent solution and given subcutaneously.

The effect in the control dog of 20 mg. of codeine phosphate per kilogram was drowsiness and very rapid and irregular respiration. The pupil was constricted. Pulse rate and temperature were slightly increased. One hour and forty minutes after the injection the dog was about as usual. Forty milligrams of codeine phosphate per kilogram caused marked disturbance of equilibrium, increased spinal reflexes, and very rapid respiration. The pupil was dilated, and the pulse rate was increased. One hour and forty minutes after the injection the dog was sleeping lightly and five hours after injection was still slightly depressed.

In the dog habituated to morphine 40 mg. of codeine phosphate per kilogram of weight caused an increase in spinal reflexes and rapid, irregular respiration. Pupil and pulse rate were unchanged. Temperature was slightly increased. Gait was normal. At the expiration of one hour and forty minutes the dog was practically normal. The larger doses of codeine in the morphine habituated dogs caused unstable equilibrium, an increase in spinal

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reflex activity, very rapid respiration, dilatation of the pupil, an increase in pulse rate and a rise of temperature. The dog which received 100 mg. of codeine phosphate per kilogram had a convulsion forty minutes after the drug was administered. One hour and forty minutes after injection both were practically as usual.

These results show a decided increase in tolerance to codeine as the result of habituation to morphine. A comparison of the effect of the 40 mg. dose in the two cases brings out clearly the difference as regards narcotic action. In the control dog this dose caused very definite disturbance of equilibrium and sleep. In the morphine tolerant dog there was no effect on equilibrium and no drowsiness. The protocols of these two experiments follow.

Normal Dog

Dog 1—male, 22.25 kg.

May 12, 1926

10:35 A.M. Lively
Pupil 9 mm.
Pulse 100
Respiration 23
Temperature 39.6°

10:40 A.M. Codeine phosphate (5% solution) 40 mg. per kilo.

10:49 A.M. Unsteady on legs

10:55 A.M. Lying down

11:02 A.M. Panting

11:07 A.M. Disinclined to walk. Marked disturbance of equilibrium. Salivated.

11:13 A.M. Spinal reflexes very active
Pupil 11 mm.
Pulse 126
Respiration 60
Temperature 39.5°

11:23 A.M. Respiration 208

12:20 P.M. Gait improved
Sleeping lightly

3:15 P.M. Only slightly depressed

Morphine Dog

Dog 99—female, 14.3 kg.

May 13, 1926

10:02 A.M. Very active
Pupil 11 mm.
Pulse 122
Respiration 15
Temperature 39.1°

10:06 A.M. Codeine phosphate (5% solution) 40 mg. per kilo.

10:39 A.M. Marked increase in spinal excitability on stimulation
Pupil 10 mm.
Pulse 122
Respiration 34, irregular
Temperature 39.5°

10:40 A.M. Vomited
Gait practically normal

11:55 A.M. Practically normal

3:00 P.M. Same

None of the morphine tolerant dogs was caused to sleep or even rendered drowsy by codeine. To produce the other effects in like degree in the morphine tolerant dogs as in the nontolerant, the dose had to be doubled.

Heroine.—Two normal dogs were given heroine on two successive days. A 0.1 per cent solution of the hydrochloride in normal saline was prepared and given hypodermically. On the first day each dog received 0.3 mg. per kilogram of body weight and on the second day 0.6 mg. The only noticeable effect of the smaller dose was on respiration, which became rapid and irregular. The larger dose caused very rapid and irregular respiration and sleep.

Our three morphine tolerant dogs were given doses of heroine ten times as large as those given to the nontolerant dogs without any effect upon respiration. One dog slept lightly after 3 mg. of heroine hydrochloride per kilogram but did not go to sleep after 6 mg. The others showed no depression with either dose.

All of the dogs, both tolerant and nontolerant to morphine, failed to show any effect upon gait or spinal reflexes with the doses used. Pulse rate and temperature were either not affected or were slightly decreased. The pupil was unaffected. A typical protocol from each group is submitted.

Normal Dog

Dog 2—female, 17.5 kg.

May 11, 1926

10:08 A.M. Pupil 12 mm.
Pulse 132
Respiration 70
Temperature 40.3°

10:10 A.M. Heroine hydrochlor.
(0.1% solution)
0.6 mg. per kilo.

10:20 A.M. Drowsy

10:45 A.M. Sleeping
Gait unaffected
Pupil 11 mm.
Pulse 104
Respiration 130 and irregular
Temperature 39.5°

12:20 P.M. Almost normal

1:30 P.M. Depression very slight

5:00 P.M. Normal

Morphine Dog

Dog 42—male, 18.25 kg.

May 11, 1926

10:20 A.M. Pupil 5 mm.
Pulse 105
Respiration 17
Temperature 39.2°

10:23 A.M. Heroine hydrochlor.
(0.1% solution)
6 mg. per kilo.

10:53 A.M. Very little, if any, depression
Pupil 6 mm.
Pulse 80
Respiration 16
Temperature 38.6°

1:30 P.M. Running about
No depression

5:00 P.M. Same

Scopolamine.—Scopolamine hydrobromide in 1 per cent solution was administered to three normal dogs. The dose was 0.67 mg. per kilogram of body weight. The only effect apparent was moderate depression. When the same dogs received the same dose of scopolamine followed thirty minutes later by morphine sulphate, 10 mg. per kilogram, they went to sleep twenty minutes after the morphine and could be roused only partially five hours later. The next day they still showed some depression. Ten milligrams per kilogram of morphine sulphate alone caused two of these dogs to vomit and all to sleep lightly. They went to sleep in twenty minutes and were drowsy five hours later

When the morphine tolerant dogs were given the same dose of scopolamine hydrobromide, 0.67 mg. per kilogram, they first became restless. Then they manifested unsteadiness in gait, walked with legs stiff, and failed to avoid objects. One became quite crazy, howling and barking and bumping into tables and the wall. Thirty minutes after the scopolamine each received his usual dose of morphine. In all depression was much more marked than usual, though they could be roused. Two vomited. All were as usual the next day.

The two protocols given below are indicative of the results obtained with scopolamine and morphine.

Normal Dog

Dog 1—male, 22 kg.

May 8, 1926

9:42 A.M. Lively
Pupil 11 mm.
Pulse 128
Respiration 27
Temperature 39.8°

Morphine Dog

Dog 99—female, 13.5 kg.

May 8, 1926

9:28 A.M. Quiet. Trembling
Slightly irritable
Pupil 10 mm.
Pulse 136
Respiration 12
Temperature 39.0°

9:45 A.M. Scopolamine hydrobromide (1% solution) 0.67 mg. per kilo.
10:12 A.M. Depression slight
A little restless
Pupil 12 mm.
Pulse 196
Respiration 130, panting
Temperature 39.8°
10:15 A.M. Morphine sulphate (2% solution) 10 mg. per kilo.
10:22 A.M. Unsteady on feet
Panting
10:23 A.M. Lying quietly
10:27 A.M. Hard to rouse
10:32 A.M. Sleeping
10:39 A.M. Sleeping
Pupil 12 mm.
Pulse 184
Respiration 180
Temperature 38.8°
12:00 M. Rouses slightly on strong stimulation
3:00 P.M. Can be partially roused

May 9

11:00 A.M. Still some depression

9:32 A.M. Scopolamine hydrobromide (1% solution) 0.67 mg. per kilo.
9:43 A.M. Restless
9:45 A.M. Unsteady on feet
Stiff-legged gait
Does not seem to know where she is going
9:50 A.M. Considerable depression
10:00 A.M. Pupil 12 mm.
Pulse 172
Respiration 30
Temperature 39.2°
10:03 A.M. Morphine sulphate (5% solution) 150 mg. per kilo.
10:10 A.M. Very marked depression
10:27 A.M. Depression much more marked than usual
Twitching
Pupil 13 mm.
Pulse 200
Respiration 110
Temperature 38.8°
10:48 A.M. Vomited
12:00 M. Rouses slightly on strong stimulation
3:00 P.M. Moderate depression
Easily roused

May 9

11:00 A.M. As usual

Cushny[2] states that dogs which have been rendered tolerant to morphine are equally refractory to its allies, codeine and heroine. Blair[3] expresses the belief that morphine and heroine addicts show increased tolerance to scopolamine. Myers[4] habituated dogs to morphine, 10-30 mg. per kilogram, and found that they showed marked tolerance to heroine and codeine as regards effect on the respiratory center, slight tolerance to the same drugs as regards the tissues governing equilibrium, and negligible tolerance as regards heart and intestinal peristalsis. He found no evidence of cross tolerance to chemically dissimilar drugs, cannabis indica and chloral hydrate. Biberfeld[5] concludes that the habituation to morphine obtained through long-continued injections is specific; it does not exist even against heroine. In our opinion Biberfeld's conclusion is not justified by his protocols. He gave heroine to two normal dogs in doses of 5.9 and 26 mg. per kilogram, respectively. In both, cerebral depression and ataxia occurred, which lasted seventy-five minutes after the smaller dose and more than three hours after the larger dose. A dog habituated to 147 mg. of morphine per kilogram received 14.7 mg. of heroine per kilogram. No ataxia resulted and only very slight cerebral depression. Another dog habituated to 97 mg. of morphine per kilogram received

35.7 mg. of heroine per kilogram. Brief ataxia and slight cerebral depression were noted. It is upon these results that Biberfeld bases his conclusion. In other experiments this author shows definitely that dogs habituated to morphine react to scopolamine alone or in combination with morphine in exactly the same way as normal dogs.

CONCLUSIONS

1. Dogs habituated to the daily subcutaneous injection of morphine, 150 mg. per kilogram, showed tolerance to codeine and heroine.

2. Dogs habituated to the daily subcutaneous injection of morphine, 150 mg. per kilogram, did not show tolerance to scopolamine.

We are indebted to Mr. J. P. Quigley for assistance in making the observations recorded in this report.

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LABORATORY METHODS

SOME NEW AND IMPROVED TESTS FOR MORPHINE AND RELATED ALKALOIDS*

By Charles C. Fulton, Omaha, Nebraska

IN THE following articles some new tests for morphine are described, and some newly discovered features of older tests, or improved methods of performing them. There are, to be sure, plenty of good tests for morphine already well known. It is one of the easiest of all alkaloids to identify. Yet some of these new tests should prove desirable additions to any list of the qualitative reactions of morphine. Attention is directed particularly to the *modified iodic-acid test*, the reaction with *nitric-acid formaldehyde reagent "A,"* and the *derivative-oxidation test*. These especially are useful both for routine identification work (as in the Laboratories of the Prohibition Bureau) and for the more difficult task of identifying suspected morphine isolated from complex mixtures in a search for drugs or poisons.

ALKALOIDS USED

The reactions described in the following articles are concerned almost exclusively with morphine and the closely related alkaloids, heroine, codeine, and dionine. There are also numerous references to the reactions of apomorphine. The alkaloids used in investigating these reactions, with the source of each, were as follows:

Morphine hydrochloride—from samples.
Morphine sulphate—Mallinckrodt.
Diacetyl-morphine hydrochloride—Mallinckrodt.
(Heroine hydrochloride)
Codeine sulphate—Mallinckrodt.
Codeine (free alkaloid)—New York Quinine and Chemical Works.
Dionine hydrochloride—Merck.
(Ethyl-morphine hydrochloride)
Apomorphine—in $\frac{1}{60}$ grain tablets.

Both the sulphate and the hydrochloride of morphine, the sulphate of codeine and the free alkaloid, were used in studying these tests. In addition, the new tests described in Articles I, II, and V have been tested on free crystalline morphine (prepared from the hydrochloride), and on morphine and codeine which I extracted from opium.

In connection with the new reagents described in Article II, the following other opium alkaloids were used:

*From the Prohibition Branch Laboratory, Treasury Department, Omaha, Nebraska. Received for publication, September 25, 1927.

Narceine—Merck.
Narcotine—Eimer and Amend.
Papaverine—Merck.
Thebaine—Merck.
Oxydimorphine—prepared from the morphine hydrochloride by oxidation with permanganate.

These other opium alkaloids, except oxydimorphine, were also tried with the tests of Articles I and V. I did not feel sure that the oxydimorphine was entirely free from impurities.

The unrelated alkaloids, atropine, brucine, cinchonine, cinchonidine, cocaine, emetine, quinine, and strychnine, were also tried with the tests of Articles I, II, and V, but no effects of any importance were discovered. The unrelated alkaloids gave little or no color with the reagents described in Article II.

ACID USED

Numerous references are made in the articles to "pure concentrated" sulphuric acid, so that it is necessary to define these terms. "C. P." sulphuric acid was used, manufactured by the General Chemical Company. The analysis on the label was as follows:

Specific gravity	— 1.84
Acidity	— 95% H_2SO_4
Fe	— 0.00001%
HCl	— "Nil"
As	— 0.0000005%
Nitrogen	— "Nil"
Nonvolatile	— 0.0009%

I. MODIFIED IODIC-ACID TEST

The iodic-acid ammonia test is well known. The morphine in aqueous solution is oxidized by the addition of a few drops of iodic acid solution. This produces, unless the morphine solution is very dilute, a yellow to yellow-brown color, not all of which is due to the iodine set free. Addition of ammonium hydroxide causes the color to become dark mahogany brown. This test is said to be very distinctive (Fuller: *Chemistry and Analysis of Drugs and Medicines*).

The modification consists in adding a few drops of hydrogen peroxide solution before the ammonia is added. Then instead of dark brown, a bright red color is produced. This red color appears more quickly than the brown, but gradually fades. The test is sensitive and is probably just as characteristic in this form as the unmodified iodic-acid ammonia test. It may be applied directly to a fairly dilute aqueous extract of opium, as there is no difficulty in recognizing the red color in such a light brown solution.

Removal of the free iodine by shaking with chloroform or carbon tetrachloride, before adding the ammonia, does not affect the reaction. The red color is not extracted by any immiscible solvent thus far tried. Other oxidizing agents may be used instead of iodic acid, but no other has been found that gives quite as good results. Codeine does not give the test.

II. NEW REAGENTS FOR THE OPIUM ALKALOIDS

If, in testing morphine, the nitric acid test and the sulphuric-acid formaldehyde test are performed on adjacent spots of a spot plate, the nitric acid test being performed first, it often happens that the fumes from the concentrated nitric acid so affect the morphine on the adjacent spot that blue and green colors are obtained with the Marquis' reagent (sulphuric acid and formaldehyde), in addition to the usual red-purple color.

Now it is easily possible to prepare a reagent, using nitric acid, formaldehyde, and sulphuric acid, which will give blue and green colors with morphine and closely related alkaloids, practically to the exclusion of the usual purple colors given with Marquis' reagent.

Put 1 drop of concentrated HNO_3 in a dry test tube and add 4 or 5 drops of 40 per cent formaldehyde solution and then about 10 c.c. of concentrated H_2SO_4. A violent reaction takes place as the first H_2SO_4 is added. When the effervescence has subsided, the reagent is ready for use. The formaldehyde must be in excess of the HNO_3, and may be considerably in excess without greatly changing the reagent. The exact amount of H_2SO_4 used is not material.

This reagent gives with morphine a momentary purple color, quickly blue-green, changing gradually to dark blue, and finally becoming dark, dirty purplish-brown-red. Codein gives olive, changing to bright green, which gradually becomes very dark green. These blue and green colors are quite intense.

This reagent will not keep. The first noticeable changes are that morphine gives a momentary brownish instead of a purple color, and blue colors appear in the codeine reaction. If simply allowed to stand in the test tube, the reagent decays to uselessness in something like a week, but if kept in a glass-stoppered pycnometer bottle and a drop of 40 per cent formaldehyde solution added each day that it is to be used, it may be preserved in practically its original condition for a long time.

This reagent in its decay passes through a well-marked stage in which the reactions given by morphine and codeine are entirely different. It is not difficult to obtain this stage in a few minutes by dilution with water; consequently we have here a second reagent which may be of value in some cases. The reagent already described may be called Reagent A, and this second one, prepared from it by dilution, may be called Reagent B.

It is somewhat difficult to prepare Reagent B with uniform success, and it should always be tested on known morphine and codeine (both) before being tried on an unknown. However, the following procedure should give good results at least four times out of five: Put 1 drop concentrated HNO_3 in a dry test tube; follow with 3 drops 40 per cent formaldehyde solution and 1 c.c. concentrated H_2SO_4. Cool in running water for a moment; add 1 c.c. water; cool; then add 4 c.c. concentrated H_2SO_4 and mix thoroughly, cooling with running water. This reagent then should give with morphine a brown color, changing gradually to dull purplish or violaceous red; and with codeine an olive-brown, changing to olive-blue-green and gradually to deep bright blue, becoming slowly purple-blue.

REACTIONS OF THE OPIUM ALKALOIDS WITH THE NITRIC-ACID FORMALDEHYDE REAGENTS

REAGENT A

Morphine: Momentary purple, quickly *blue-green*, gradually *dark blue*, and finally dirty purplish-brown-red.
Codeine: olive changing to *bright green*, gradually very *dark green*.
Heroine: like morphine.
Dionine: like codeine.
Apomorphine (fragment of 1/20 grain tablet): *purple* changing to dull dark *green*, finally brownish-red-purple.
Oxydimorphine: olive-green changing to strong *bright bluish-green*.
Papaverine: the solid turns purple-brown before dissolving; on solution there is little color at first, but a *deep bright blue* soon develops.
Narcotine: yellow before dissolving, then *dirty violet-red*, changing through brown to *bright red*.
Narceine: dark yellowish-brown changing to orange, then red, then brown (about the same as with Marquis' Reagent).
Thebaine: red-brown before dissolving, then bright orange-red changing to brownish-orange (about the same as with pure concentrated H_2SO_4).

REAGENT B

Morphine: *brown*, changing gradually to dull purplish or violaceous *red*.
Codeine: olive-brown changing to olive-blue-green and gradually to *deep bright blue*, becoming slowly purple-blue.
Heroine: same as morphine.
Dionine: same as codeine.
Apomorphine (fragment of 1/20 grain tablet): dirty *green* changing to *blue* and slowly becoming *purple*.
Oxydimorphine: brown, changing slowly to light blue green, then to green.
Papaverine: slight gray, gradually deep bright *blue*.
Narcotine: yellowish, changing gradually to bright *carmine-red*.
Narceine: yellow-brown, then with a greenish tinge; then slightly purplish at edge, brownish solution; finally brownish-orange. (Colors not strong, not distinctive.)
Thebaine: bright orange-red.

III. PELLAGRI'S TEST

Pellagri's test for morphine consists in converting it to apomorphine, and detecting the latter by the strong, bright colors of its oxidation product. This is a well-known test which is discussed here because of its relation to the "derivative-oxidation test," described under V, following.

The directions usually given for Pellagri's test (for instance by Autenrieth-Warren: *The Detection of Poisons*) are as follows: Dissolve a little morphine in about one-half c.c. of concentrated HCl and add a drop or two of concentrated H_2SO_4. Evaporate off the HCl at from 100° to 110° C., or on the water-bath. Take up the H_2SO_4 residue with water, add $NaHCO_3$ in excess, and oxidize with a drop or two of iodine in alcohol, avoiding excess. The aqueous solution is emerald green, and ether extracts pink or red.

A method which is much more convenient and better in every way is as follows: Put a little dry morphine in a test tube and dissolve in a small amount of syrupy phosphoric acid (85 per cent H_3PO_4). Heat to boiling over a small free flame. Cool, dilute with a few c.c. water, and oxidize by adding several drops of HIO_3 or KIO_3 solution (5 per cent). An orange-red color is produced. Add considerable ammonium acetate, and the color changes through olive to bright green or blue-green. Ether and other organic solvents then extract the colors characteristic of the oxidation product of apomorphine. The advantage of NH_4Ac over $NaHCO_3$ is that the former does not cause foaming when it is added to the acid solution.

IV. DERIVATIVES FORMED ON HEATING IN PURE CONCENTRATED SULPHURIC ACID

Morphine.—When morphine is dissolved in concentrated sulphuric acid, it begins at once to undergo an essential change. This change is made complete in a few minutes by warming the solution to about 40° C. The sulphuric acid solution remains colorless, but various tests show that the morphine has been completely converted to some entirely different compound. This new substance has the following properties:

a. Not very soluble in cold water or cold dilute acid. On diluting the concentrated H_2SO_4 solution with say 10 times as much water (or more), and allowing it to stand, the compound crystallizes out as white transparent needles or rods, or small rods with rounded ends, often in rosettes. Very soluble in alkalies; soluble in concentrated H_2SO_4; may be recrystallized from hot water or hot dilute acid.

b. Does not give precipitates from the dilute acid solution (tested before the compound has crystallized out) with such general alkaloidal reagents as Wagner's, Mayer's, phosphomolybdic acid, etc. Thus it appears that the morphine has been converted into some substance which is not even an alkaloid.

c. Shows close similarity to apomorphine in its color reactions. In fact, these are for the most part identical; concentrated HNO_3, for instance, gives exactly the same succession of colors as with apomorphine: red-purple to red to orange. But there are some differences, notably in regard to the different solubilities of the oxidation product. (See derivative-oxidation test.)

d. Like apomorphine a strong reducing agent; more easily oxidized than morphine.

Of course the resemblance of the color reactions of this compound to those of apomorphine is not accidental. The two must be nearly alike in constitution. It may be that the only difference is one of polymerization.

It will be noted that the sulphuric acid solution is to be heated only to about 40° C. to produce the compound under discussion. If it is heated too hot, say to 100°, the derivative will not crystallize out as before, and various color reactions show that there has been a further change. A study of the color reactions shows that this further change takes place at from 60° to 70° C. For instance, the color reactions obtained by adding a tiny crystal of KNO_3, or a few grains of NH_4 persulphate, to the H_2SO_4 solution, and the amount of color extracted from the green solution of the oxidation product by amyl alcohol, all show a change at about the same point. Table I shows the observed results:

TABLE I

TEMP. ° C.	H_2SO_4 SOLUTION		AMYL ALCOHOL EXTRACT
	KNO_3	NH_4 PERSULPHATE	
20°	orange-red to purple to gray blue to green	olive to green	good blue
30°	"	"	strong blue
40°	"	green	"
50°	"	"	
60°	orange-red to purple to purple-brown	olive to yellow-brown	good blue
70°	orange-red to dull purplish red to purplish red-brown	"	weak blue
80°	intense orange-red to dark red	orange-brown	faint blue
90°	"	"	no color
100°	"	"	"

From 100° to about 140° C. the reactions are practically unchanged. At the latter temperature, or somewhat above it, a dark olive-green color begins to develop in the concentrated sulphuric acid solution. This sulphuric-acid color test merits separate discussion.

That morphine is converted into a compound resembling apomorphine in its color reactions when heated in concentrated sulphuric acid has, of course, long been known. Husemann's test is the application generally given. The degree of heat necessary has been greatly exaggerated, however, and so far as I have been able to learn, the production of a crystalline derivative insoluble in cold water or dilute acid on heating only to 40° C. has been entirely overlooked. The derivative-oxidation test described in the following article also seems to be new; and certain other tests, easily made on this morphine derivative, have been given little attention, notably the derivative-iron test.

Codeine.—When codeine is warmed in concentrated sulphuric acid, it is converted into a different substance in the same manner as morphine. The two derivatives are not the same. The codeine derivative retains its distinctive qualities until the sulphuric acid solution in which it is formed has been heated at least to 100° C.

On dissolving codeine in concentrated sulphuric acid and warming to 40°, or at the most, 50° C., a crystalline derivative is formed having the following properties:

a. Not very soluble in cold water or cold dilute acid; soluble in alkali or concentrated H_2SO_4. Crystallizes out as transparent white needles or rods in sheaves, rosettes, or spheres. Sometimes with considerable codeine soon after diluting the H_2SO_4 solution with water, the whole aqueous solution sets to a white jelly, in which the crystals gradually form as spheres of needles, like little cockle-burs, the jelly gradually disappearing.

b. Like the corresponding morphine-derivative, it does *not* give precipitates with the general alkaloidal reagents from the dilute acid solution.

c. Color reactions similar to those of apomorphine, and more closely similar to those of the corresponding morphine-derivative, but in some reactions distinctly different from either. Thus, this codeine-derivative is like the morphine-derivative and different from apomorphine in the solubilities of the colored oxidation product, and is different from both in not giving any color reaction in the iron test.

d. A good reducing agent, though codeine itself is not considered a reducing agent at all. But it is not so easily oxidized as the corresponding morphine-derivative, and the latter not so easily as apomorphine.

With codeine, as with morphine, a further change takes place at about 60° to 70° C., as shown by the color reactions. The change is of the same nature as with morphine.

At about 100° C. on heating in concentrated sulphuric acid, the codeine-derivative begins to lose the peculiarities which distinguish it from the corresponding morphine-derivative.

At from 140° to 150° C., as with morphine, a dark olive or olive-green color develops, and the same sulphuric-acid color test is obtained.

Heroine.—So far as I have discovered, heroine reacts exactly like morphine when heated in pure concentrated sulphuric acid. This is natural, because of the ease with which heroine is hydrolyzed to morphine.

Dionine.—The dionine reaction is practically the same as that of codeine. Such differences as there are show that dionine is intermediate between codeine and morphine in its chemical character. Thus, dionine begins to give the iron test after heating to only about 70° in concentrated sulphuric acid, while codeine requires 100°. This fact may be used conveniently to distinguish between dionine and codeine. The test will be described in the next article.

EXACTNESS OF THE TEMPERATURES STATED

Many, perhaps all, of the changes produced immediately at a certain temperature are effected gradually at lower temperatures. When morphine is dissolved in concentrated sulphuric acid and the solution allowed to stand overnight (as in some directions for Husemann's test), it is changed more than when heated within a few minutes to from 40° to 50°. In following procedures given in this and subsequent articles, the test tube containing the alkaloid freshly dissolved in concentrated sulphuric acid is to be placed in a suitable bath and heated steadily, with sufficient rapidity or slowness that the contents of the tube will be heated *to*, rather than *at*, the temperature shown by the thermometer in the bath.

COMPARISON OF COLOR REACTIONS

In Table II a comparison is given between the color reactions of apomorphine and those of the morphine and codeine derivatives, formed by heating in pure sulphuric acid to from 40° to 50° C.

Erdman's reagent does not give strong, well-marked colors; the reactions described for this reagent are not to be considered very reliable.

TABLE II

	APOMORPHINE	MORPHINE DERIVATIVE (HEROINE-DERIV. SAME)	CODEINE-DERIVATIVE (DIONINE-DERIV. PRACTICALLY THE SAME)
Concentrated nitric acid	red-purple changing to red and then to orange	red-purple changing to red and then to orange	red-purple changing to red and then to orange
Froehde's reagent	bright green	bright green	bright green
Marquis' reagent	purple changing to green-black	purple changing to green-black	yellowish, brownish, then deep green develops
Erdman's reagent	green changing to purple	purple changing to green-blue, finally dull green	orange changing to blue-green then to dull blue
NH_4 persulphate to H_2SO_4 sol.	develops olive brown changing to dull green	develops yellowish green changing to green	develops bluish green changing to green
Oxidation test	orange-red in acid sol.; green in neutral or slightly basic sol.; amyl alc. ext. blue; ether purplish red; chloroform purple	orange-red in acid sol.; green in neutral or slightly basic sol.; amyl alc. ext. blue; ether and chloroform ext. no color.	orange-red in acid sol.; green in neutral or slightly basic sol.; amyl alc. ext. blue, ether and chloroform ext. no color.
Iron test	NH_4Ac — light purplish blue, Na_2CO_3—red	NH_4Ac — light purplish blue, Na_2CO_3—red.	no reaction

V. THE DERIVATIVE-OXIDATION AND DERIVATIVE-IRON TESTS

The "derivative-oxidation test" and the "derivative-iron test" may be conveniently performed at the same time. Both depend on the fact that morphine heated in concentrated sulphuric acid to from 40° to 50° C. changes into a compound giving color reactions similar to those of apomorphine.

Procedure.—Put a little morphine or its salt in a clean dry test tube and dissolve it in from 12 to 16 drops of concentrated H_2SO_4. Stand the tube in a beaker of water with a thermometer and warm to from 40° to 50° C. (not over 60° C.). Remove tube and add 3 or 4 c.c. of water. Divide the solution into two portions for the 2 tests:

1. Derivative-Oxidation Test. This is given by morphine, heroine, codeine, and dionine. Add 3 or 4 drops 5 per cent HIO_3 solution. An orange-red color develops. (Bright red with considerable morphine, light orange with very little.) The solution may be heated to hasten the oxidation. Heating it to boiling will do no harm, and ensures complete oxidation. However, it is scarcely necessary to heat with morphine or heroine, but heating is advisable with codeine or dionine. Add considerable NH_4Ac, or an excess of $NaHCO_3$. The latter is preferable except for the foaming it causes, which is sometimes very troublesome. The color changes to bright green or bluish-green. If a larger volume of solution is desired, additional water may be added after oxidation, either before or after destruction of the excess of acid. The solution to which the HIO_3 is added should contain about 4 drops of concentrated H_2SO_4 per c.c., particularly for oxidation of the codeine-derivative. The derivative of morphine is more readily oxidized. Shake the green solution with a little amyl alcohol. Most of the color, or even all of it, goes into the amyl alcohol, which becomes blue.

2. Derivative-Iron Test. This test is given by morphine and heroine, but not (at the temperature stated) by dionine or codeine. Narcotine reacts to the test, though not quite the same as morphine. Add 1 or 2 drops of quite dilute $FeCl_3$ solution. (Say about 0.2 per cent. Dilute 1 drop of 10 per cent $FeCl_3$ with 1 c.c. of water for this purpose.) Or a drop of $FeSO_4$ solution may be used instead. Add Na_2CO_3 in excess. Just as the excess of acid is destroyed, a blue color suddenly develops, changing at once to purple, and then with the excess of Na_2CO_3 it becomes and remains red.

A few additional facts about each of these tests may now be given:

DERIVATIVE-OXIDATION TEST

This is believed to be an entirely new test, but its resemblance to Pellagri's is obvious. The green color in practically neutral or slightly alkaline solution and the blue in amyl alcohol are the same. In this test, however, ether or chloroform cannot be substituted for amyl alcohol, as neither one extracts any color at all. (Except that due to the iodine set free, not noticeable with such quantities of morphine as will ordinarily be used for this test.) In fact, the only organic solvents thus far discovered which will dissolve out this green color are the lower alcohols and acetone. Isopropyl alcohol and acetone extract the color from a solution saturated with salt; ethyl and methyl alcohols, used in conjunction with chloroform, also extract it from a satu-

rated salt solution, and normal butyl alcohol extracts it. These solvents all become blue. Caprylic alcohol does not extract the color.

This oxidation product is therefore sharply distinguished from the oxidation product of apomorphine by its failure to dissolve in most organic solvents. The oxidized apomorphine dissolves in all kinds of organic solvents with red, blue, purple, and violet colors. This other oxidation product of dehydrated morphine shows only blue and green colors, but as the oxidized apomorphine is also blue in the lower alcohols, the only actual difference of color is in acetone. In this solvent the apomorphine oxidation-product is blue with a distinct purple tinge, while the other is at first simply blue, then greenish-blue, and on standing turns to green and then to yellowish.

As already mentioned, the codeine-derivative is not so easily oxidized as the morphine-derivative, and the latter not so easily as apomorphine. This is shown by the relative ease with which iodic acid produces an orange-red color in a solution only slightly acid. The dionine-derivative is in between those of codeine and morphine; heroine reacts just like morphine. The derivatives of morphine, codeine, and dionine (formed by warming in concentrated sulphuric acid) seem to give the same oxidation product.

The oxidation product of apomorphine is less easily reduced than the other. The color change of the oxidized morphine-derivative in acetone has already been described. This is doubtless due to reduction. The oxidized apomorphine shows a similar change when treated with a strong reducing agent. For instance, on treating its blue solution in amyl alcohol or its red solution in ether, with a drop of stannous chloride solution, the color is changed to green and then to light yellow or colorless. (Wangerin's test.)

If to the green solution of the oxidized morphine-derivative considerable concentrated ammonia is added, the color is changed gradually to purplish-blue. Amyl alcohol readily extracts all the color from this solution, and becomes purple.

If the solution of the alkaloid in concentrated sulphuric acid is heated too hot, say to 100° C., the orange-red color is produced on oxidation and the green on subsequent neutralization, just as described for the test, but neither amyl alcohol nor any other immiscible solvent thus far tried will then extract any color at all.

DERIVATIVE-IRON TEST

This test is not new, though it has scarcely been described in the form given here. (See Perkin: *Qualitative Chemical Analysis.*)

If $NaHCO_3$ is substituted for Na_2CO_3, the final color is a purplish-pink. NH_4Ac gives light purplish-blue. NH_4OH gives sometimes violet and sometimes red, or an intermediate color. These colors are not extracted by amyl alcohol nor by other immiscible solvents. If considerable NH_4OH is added and the solution is allowed to stand overnight, the color is then more of an orange-brown by transmitted light, and on looking obliquely down the tube a strong and beautiful purple fluorescence is seen.

The ferric chloride may be added either before or after the excess of acid is destroyed. If the carbonate or bicarbonate is added first, a slight pink color

is generally produced, due to a trace of iron in the reagent. The reaction may be used as a sensitive test for iron in the sulphuric acid, the water, or the substance used to destroy the acidity. In fact, an excessive amount of iron in the magnesium oxide of this laboratory was discovered through experimenting with this test.

Distinguishing Dionine from Codeine.—This iron test provides a simple and convenient means of distinguishing dionine from codeine. The latter gives the test only after heating in concentrated sulphuric acid to above 100° C., while dionine reacts after heating to above 70° or 75°, and may even give a very faint test after heating only to 60° or 50°. Dissolve the alkaloid or its salt in a little concentrated H_2SO_4, heat to 80° or 90° C., dilute, add 1 or 2 drops of dilute $FeCl_3$, and Na_2CO_3 in excess. If a good red color is obtained, the alkaloid is dionine; if the color is only light yellowish or brownish, it is codeine. By testing suspected dionine in comparison with known codeine, or vice versa, it would scarcely be possible to make a mistake. So far as morphine and heroine are concerned, practically the same test is obtained after heating in concentrated H_2SO_4 to anywhere from 30° to 140° C.

Reaction of Narcotine.—Narcotine also changes its properties when warmed in concentrated sulphuric acid, the change being probably one of hydrolysis rather than dehydration as with morphine. One of the new properties is that of giving a reaction in the iron test. With sodium carbonate a brownish-red color is obtained, which soon resolves itself into a reddish-brown precipitate and a light pink solution. Ammonium acetate gives a light grayish-blue color.

The Iron Test in Conjunction With Pellagri's Test.—Apomorphine, without any heating in acid, gives the colors we have described for the iron test. Solvents may extract some of the color, however. In particular, amyl alcohol extracts the color from the ammoniacal solution, becoming brownish-purple. So the iron test may be used along with Pellagri's test, as well as with the derivative-oxidation test. Such use is less convenient, however. With only a moment's boiling in phosphoric acid to produce the apomorphine, it will be found that morphine generally gives the iron test quite well, dionine faintly, and codeine not at all, while all give Pellagri's test quite well. Thus, it appears that apocodeine does exist, and if it is a mixture, it does not necessarily contain apomorphine, as has been suggested. With longer boiling in the phosphoric acid the apocodeine is broken down, the solution gives the iron test, and no doubt does contain apomorphine.

VI. HUSEMANN'S TEST

All authorities give Husemann's test as one of the most important tests for morphine, but practically every one gives a method for performing it somewhat different from that given by any of the others, and a somewhat different description of the colors produced. (Consult, for instance, Fuller: *Chemistry and Analysis of Drugs and Medicines;* Autenrieth-Warren: *The Detection of Poisons;* Mulliken: *The Identification of Pure Organic Compounds;* Allen's *Commercial Organic Analysis;* etc.) A generalized description

would seem to be as follows: Dissolve the morphine in concentrated sulphuric acid and heat this solution at about 100° C. for half an hour. Cool, and oxidize with a trace of nitric acid, or a small crystal of potassium nitrate or chlorate. A momentary purple or "violet" color is produced, changing to red and then to orange (i.e., the same succession of colors as is given by apomorphine with concentrated nitric acid).

The test works all right, except that the momentary purple or violet color is seldom, if ever, obtained. A blood red or dark red color is produced at once, when the oxidizing agent is added, unless the sulphuric acid is first diluted slightly. If a drop of water is added to 6 or 8 drops of the sulphuric acid solution, and then a small crystal of potassium nitrate stirred in, the purple-red-orange succession of colors may be obtained. The best description of Husemann's test that I have found is that given by Fresenius-Wells in their *Qualitative Chemical Analysis;* it suggests (to my mind) that the "concentrated sulphuric acid" formerly used was weaker than that in use today. Confusion may have arisen, however, through misuse of the word "violet," a color-term much abused in chemical literature. The solution of the oxidation product of apomorphine in ether is sometimes spoken of as "violet" or "reddish-violet," though it is simply pink when dilute, and nearly crimson when concentrated.

Heating the sulphuric acid solution for half an hour at 100° discolors it more or less; if fairly well exposed to the air it becomes dirty violet. Since morphine dissolved in concentrated sulphuric acid is completely changed to a compound giving color reactions similar to those of apomorphine by merely warming to 40°, most of this heating would seem to be unnecessary, and even unwise, especially if impurities are present. There are, however, differences in the reaction if the solution is heated only to 40° rather than to 100°; but the 40° reaction is probably just as important and interesting as the other; heating to 100° suffices for the other, and this may be done in five or ten minutes just as well as in half an hour. Incidentally, the reactions are more like those of apomorphine after heating only to 40°.

A test will now be described which includes several reactions making use of the sulphuric acid solution (containing the derivative of morphine) after heat treatment. The effects of ammonium persulphate and formaldehyde are included here for convenience, though the persulphate gives a different sort of oxidation from the nitrate or the chlorate, and the formaldehyde reaction is of course entirely different.

Dissolve a small amount of dry morphine or its salt in about 1 c.c. of pure concentrated H_2SO_4, place the test tube in a beaker of water with a thermometer, and heat to 40° or 50° C. Draw out about half the acid, putting from 6 to 10 drops on each of five spots of the spot plate. Continue heating the remaining acid solution.

The acid solution which has been heated to 40° or 50° gives the following reactions:

HNO_3 (streak): Dip a glass thread in concentrated HNO_3 and draw it through the solution. Its path is marked by a blood-red streak, the streak being first strong orange-red, then *blood-red*, and then dark red. Meanwhile

the solution near the streak becomes orange-red changing to red, and the outer parts of the solution become first *purplish* and then bluish.

HNO_3 (trace): Add a very small trace of concentrated HNO_3 and stir it in. An orange-red color is produced which changes at once to purple and this soon to gray blue and then to dull green. A tiny crystal of KNO_3 gives the same result. Excess of KNO_3 or HNO_3 must be carefully avoided, in order to get the succession of colors stated. Otherwise the solution will probably become and remain red or orange-red.

$KClO_3$: Add a very tiny crystal of $KClO_3$ to the solution, crush, and stir it in. The crystal turns orange when added; as it is stirred in, the solution becomes *purple:* this quickly changes to gray-blue and then to *green.* If too much $KClO_3$ is used, orange and brown are the final colors produced though the purple is generally noticed easily.

$(NH_4)_2S_2O_8$: Stir in a few grains of ammonium persulphate. An olive-green or *green* color soon develops.

Formaldehyde: Add a trace of HCHO and stir in. A strong deep *purple* develops, changing to blue-black and then to green-black. (Like apomorphine.) The codeine and dionine derivatives, however, do not give purple, but develop a green color.

Heat the remaining acid solution to the temperature of boiling water, then distribute it on a spot plate. With the agents already mentioned the reactions now obtained are as follows:

HNO_3 (streak): Streak *blood-red* changing to *dark red.* The outer parts of the solution become *orange.*

HNO_3 (trace): Intense *orange-red* changing to *dark red.*

$KClO_3$: Orange-red to red.

NH_4 persulphate: Orange-brown.

Formaldehyde: Dark purple-brown.

With the exception of the formaldehyde reaction, where the difference has already been noted, morphine, heroine, codeine, and dionine all give practically the same colors in the tests.

On the whole it would seem that Husemann's test has been overrated. The derivative-oxidation test is probably better, even when the similar Pellagri's test is also used.

SUMMARY

1. A new test for morphine, not given by codeine, is described. It is a modification of the iodic-acid ammonia test, and might be called the "iodic-acid peroxide test."

2. Methods for making two new reagents for the opium alkaloids are given, and their reactions with the opium alkaloids described. These reagents are made from nitric acid and formaldehyde in solution in concentrated sulphuric acid, the formaldehyde being in excess of the nitric acid.

3. An improved method for performing the well-known Pellagri's test is described.

4. Preparation of a new derivative of morphine is described. Codeine and dionine give similar but distinct derivatives. The derivative is formed

by heating the alkaloid in concentrated sulphuric acid solution to 40° or 50° C., and is no doubt a dehydration product. On diluting with water it then gradually crystallizes out. The morphine-derivative is not apomorphine, but it gives similar color reactions.

5. Two tests based on the formation of the derivative mentioned in the preceding article are described in detail. The derivative-oxidation test is given by morphine, heroine, codeine, and dionine, and is a new test similar to Pellagri's. The derivative-iron test has been previously described, though scarcely in the form given here. It is given by morphine and heroine, but not (after heating to only 40° or 50°) by codeine or dionine. However, the test can be used to distinguish between codeine and dionine, for after heating to 80° or 90° C. it is given by dionine but not by codeine.

6. Husemann's test is discussed, with particular attention to the difference in the reactions when the sulphuric acid solution is heated only to 40° or 50° C. instead of to 100°.

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A NOTE ON THE NORMAL SERUM CALCIUM CONTENT OF MAN*

By Joseph H. Roe, Ph.D., and Bernard S. Kahn, M.D., Washington, D. C.

THE generally accepted normal blood calcium content for a man of 9 to 11 mg. per 100 c.c. of serum is based upon reports in practically all of which the Kramer-Tisdall[1] permanganate titration method was used. However, Rosen and Krasnow[2] recently reported values obtained by the latter technic which were definitely higher than the usually accepted normal. These authors found values of 10.7 to 13.2 mg. per 100 c.c. of serum in a series of analyses of sera from 50 medical students. As other controversial reports are in the literature concerning what is the normal value for blood calcium, it occurred to us that it would be of interest to carry out a series of serum calcium determinations upon normal subjects, using the colorimetric method of analysis we[3] have developed.

In our method the proteins of the serum are removed by adding 4 volumes of 10 per cent trichloracetic acid. Five c.c. of the trichloracetic acid filtrate is alkalinized until distinctly pink to phenolphthalein, 1 c.c. of 1 per cent Na_3PO_4 solution is added and, after one hour's standing, the mixture is

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centrifuged and the supernatant fluid is decanted. The mat of $Ca_3(PO_4)_2$ in the bottom of the centrifuge tube is washed twice with 50 per cent alcohol of N/0.05 alkalinity, centrifuging two minutes to throw down the $Ca_3(PO_4)_2$ in each instance. The $Ca_3(PO_4)_2$ is then dissolved in 10 c.c. of H_2SO_4 of approximately 1.4 normality. This solution and 10 c.c. of standard phosphate solution containing 0.05 mg. of phosphorus acidified to approximately 1.4 normality with H_2SO_4, are treated with 1 c.c. of 5 per cent sodium molybdate and 1 c.c. of 0.5 per cent hydroquinone in 15 per cent sodium bisulphite. The solutions are then boiled for ten minutes, cooled, and made up to a definite volume with water, usually 15 c.c. The unknown solution is then compared with the standard in a colorimeter.

In this paper we are reporting a series of calcium determinations upon the sera of fifty normal subjects. These subjects were medical students and nurses with no evidences of disturbed calcium metabolism. The values obtained in this series of analyses are shown in the table. They vary from 9.0 to 11.6 mg. per 100 c.c. of serum, the average being 10.13 mg. per 100 c.c. These findings are in accord with the generally accepted normal of 9 to 11 mg. per 100 c.c. of serum, and are of interest in that they were obtained by a new and entirely different technic.

SERIAL NO.	MG. OF CALCIUM PER 100 C.C. OF SERUM	SERIAL NO.	MG. OF CALCIUM PER 100 C.C. OF SERUM
1	10.1	26	10.1
2	9.2	27	11.4
3	10.4	28	11.6
4	9.7	29	9.7
5	9.3	30	10.2
6	9.4	31	10.3
7	9.7	32	9.9
8	10.5	33	10.6
9	9.9	34	9.9
10	9.0	35	9.8
11	9.4	36	9.5
12	9.3	37	10.0
13	9.4	38	10.3
14	9.5	39	10.4
15	10.7	40	9.7
16	10.8	41	10.3
17	11.4	42	9.7
18	10.1	43	10.4
19	11.2	44	10.6
20	9.4	45	11.1
21	9.1	46	10.7
22	11.2	47	9.8
23	9.4	48	9.1
24	10.1	49	10.8
25	11.2	50	11.2

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A NOTE IN CONNECTION WITH READING RESULTS OF THE KAHN PRECIPITATION TEST*

By Herbert Silvette, Richmond, Virginia

THE commonest complaint that one hears in regard to the Kahn test is the difficulty in reading the reaction, once it is finished. The difficulty in this case is not due to the fineness or the delicacy of the floccules, for even a one-plus test is very definite as regards the precipitation, but is caused by the kind and source of the light by which the reactions are usually read.

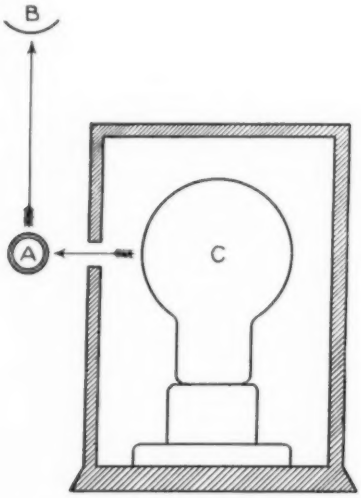

Fig. 1.—Diagrammatic representation of the ultramicroscope principle applied to reading the Kahn test. *A*, reaction tube; *B*, eye; *C*, source of light. Arrows show path of light.

Reflected, not transmitted, light should be used to judge the amount of a flocculent precipitate. As illustrated by the ultramicroscope, reflected light will reveal particles in suspension much too small to be seen by any other form of illumination. When this same principle is applied to reading the results of the Kahn test, the reaction tube is illuminated by a narrow beam of light which strikes the floccules at right angles to the line of vision from the eye to the tube. The smallest amount of flocculation then seen constitutes a doubtful or positive reaction; the negative tubes are invariably opalescent when read by reflected light.

As illustrated, the reaction tube should be held within a half inch of, and directly opposite, the aperture through which the light is issuing. The eye may be at any distance above the tube, depending upon its individual focus,

*From the Pathological Laboratory, Johnston-Willis Hospital.
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but in order to get the most perfect results, the beam of light and the line of vision must be perpendicular to each other. In explanation of this, the light passes horizontally through the liquid, and is reflected by the floccules in suspension, if present, vertically upward to the eye. Should there be no precipitate, no light is reflected; it passes in a straight line directly through the tube.

When the apparatus described is used in a dark room so that a single beam of light is all that illuminates the tube, results of the Kahn test may be reported with the fullest confidence that, in so far as the reading of the reactions goes, neither a false positive nor a false negative was obtained.

THE ISOLATION OF YEASTS AND MOLDS*

By Grace A. Hill, A.B., M.S., Belmont, California

WE HAVE found a very satisfactory medium for the isolation of yeasts and molds in the Stiritz[1] modification of Lund's[2] wort agar. Stiritz substitutes "near-beer" for the wort, because it is more easily obtainable and gives quite as good results.

Near-beer agar has the advantage of being simple to prepare. The contents of one pint-bottle of near-beer is made up to 900 c.c. with tap water. Fourteen grams of agar are added, and dissolved by boiling. It is then filtered, flasked in 100 c.c., or other known portions, and autoclaved. Before pouring the plates, 4 c.c. of sterile 5 per cent lactic acid is added to each 100 c.c. of melted agar.

The limited protein, as well as the acid reaction, serves effectually to inhibit all bacterial growth, without, apparently, having any effect upon the fungi.

I have used this medium with gratifying results in making yeast and mold counts of butter, and also in the examination of pathologic material in the clinical laboratory.

THE YEAST AND MOLD COUNT OF BUTTER

When the yeast and mold count of butter was first attempted in "butter standardization" at the University of California,† several methods, recommended by other laboratories doing this work, were tried and discarded.

In making a yeast and mold count of butter any medium on which even a few bacterial colonies develop is unsatisfactory, since it is difficult, or impossible, to distinguish between some of the smaller-celled yeasts and some of the larger bacteria, even with the low-power objective; which makes it necessary to fish and examine doubtful colonies in a wet mount; and I believe, contrary to the popular theory, that no amount of experience will enable one

*From the Laboratory of the California Sanatorium, for the Treatment of Tuberculosis. Received for publication, September 16, 1927.

†It was my duty to make the yeast and mold counts of butter, from January to August, 1923, in the College of Agriculture, Davis, California.

to determine, without a slide examination, whether a deep lenticular colony is yeast or bacteria, on plates where both yeasts and bacteria develop.

At first, in our butter examination, we used whey agar and tried varying amounts of lactic and tartaric acid. Always, unless the yeasts and molds were inhibited by the acid, some colonies of bacteria developed on these plates. When we tried near-beer agar and found that no bacterial colonies appeared, it remained to be proved whether the yeasts and molds developed as well on this medium. The first 100 samples analyzed were plated for comparison in both the standard whey and the near-beer agar. As the counts of the latter averaged about 4 per cent higher than the whey agar counts, near-beer was adopted for our routine examinations.

PATHOGENIC YEAST AND MOLDS

In our laboratory we now routinely culture, with near-beer agar, the sputum of patients suffering from respiratory diseases of unknown etiology. Also, other pathologic material, in which fungi are suspected, is either streaked or plated by this method.

In securing sputum specimens for culture, every possible precaution is taken to avoid contamination. The patient receives, with printed and oral instructions, a wrapped, sterilized container, a small bottle of glycothymoline, and a bottle of sterile, normal salt solution. Altogether, the throat is gargled and the mouth washed at least ten times.

For dilution, small, glass-stoppered bottles, containing 9 c.c. of normal salt solution and a few beads, are capped with paper and autoclaved. One c.c. of the specimen is shaken in the bottle to form an emulsion. One c.c. of this 1:10 dilution is plated in about 10 c.c. of the acidified near-beer agar. The plates are incubated at 37° C. and counted in seventy-two hours. Yeasts, however, develop in from twenty-four to forty-eight hours.

In 72 per cent of the sputum specimens examined, as is, perhaps, to be expected, a few colonies of the common molds developed on the plates. In 60 per cent of the sputum specimens examined (more unexpectedly, and worthy, I think, of further consideration), yeasts were found. Several patients having mucopurulent sputum, negative for tubercle bacilli, were found to maintain counts of these yeasts averaging about 1000 per c.c. Monilia tropicalis was isolated from the sputum of one patient. A pathogenic actinomyces and the thrush fungus were easily isolated by this method.

It is not the object of this paper to take up any particular case or organism, but to present "near-beer" agar as a very simple and satisfactory method for making yeast and mold counts of butter, or other foods, as well as for the isolation of pathogenic fungi in the clinical laboratory.

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MODIFIED WRIGHT'S TECHNIC FOR THE STANDARDIZATION OF VACCINES*

By Leland W. Parr, Ph.D., Beirut, Grand Liban, Syria

WRIGHT'S method for enumerating the bacteria present in suspensions used in the preparation of bacterial vaccines is one of the best known procedures for this purpose. Using this method[1] one draws, in order, into a capillary pipette equal volumes of sodium citrate solution, normal human blood, and the bacterial emulsion to be used in the preparation of the vaccine; each of the three equal volumes separated from the other by a bubble of air. One then empties the pipette into the concavity of a hanging-drop slide or into a watch glass and mixes the three substances thoroughly, usually by means of the pipette. After this, smears of the mixture are made on slides for appropriate staining. If on examination the stained smears are found satisfactory, a count is made to ascertain the relative number of bacteria and red blood cells, and from this it is easy to calculate the strength of the bacterial suspension.

The method requires the speed and dexterity of an expert, and the volume of the mixture so obtained is small and soon dries up if not used at once. This interferes with repeating the staining, should that be desired. As an instructor of students of medicine for the past seven years, I have not yet encountered a student who could get good results with this method without an inordinate expenditure of time and materials. More recently I have been using the method described below.

First, a small amount of citrate solution is put into a serologic tube of the sort commonly used in the Wassermann test, about 0.2 cubic centimeters being a satisfactory amount to use. Then, with a capillary pipette, as large a drop of blood as possible is taken from the available normal finger or ear. Marking the level in the pipette to which this drop of blood rises, it is then expelled into the citrate solution in the serologic tube, and by reaspiration all traces of the blood are washed from the pipette. Next, the pipette may be washed in alcohol and ether and passed through a flame if there is any question of its sterility, after which a volume exactly equal to the volume of the blood originally taken is taken up from the bacterial suspension. The bacteria are then blown out into the blood-citrate suspension, and complete homogenization is obtained by agitating the tube. I have found this to be much easier to do in this way than to obtain mixture by the usual use of the capillary pipette. When thorough mixture has been attained, slides may be made from the suspension as in other methods, but it is found more satisfactory, unless the blood drop was unusually large, to smear the mixture on the slide with a platinum loop rather than to attempt the usual blood film.

*From the Department of Bacteriology and Hygiene, American University of Beirut, Beirut, Grand Liban, Syria.

Received for publication, September 17, 1927.

[1]Kolmer: Infection, Immunity, and Biologic Therapy, Philadelphia, 1925, W. B. Saunders Co.

This method offers unusual advantages in teaching. All steps can be taken leisurely, allowing ample time for full explanation and relevant questions. The volume of the suspension is sufficient for the preparation of many slides, and it is not liable to be lost by evaporation of the diluent during an ordinary class period. For the laboratorian, also, who only occasionally makes a vaccine, the method has value, though it is not suggested that the expert would profit by changing the better known Wright's method for this modification.

A CONTAINER FOR FECES*

By E. M. Watson, M.D., M.Sc., London, Canada

ONE of the problems which not infrequently confronts the physician or laboratory worker is the adoption of a satisfactory means whereby samples of feces may be collected, sent to the laboratory, and examined with a minimum of discomfort for all concerned. Methods have been recommended with this purpose in view. For example, Einhorn[1] has recently described a

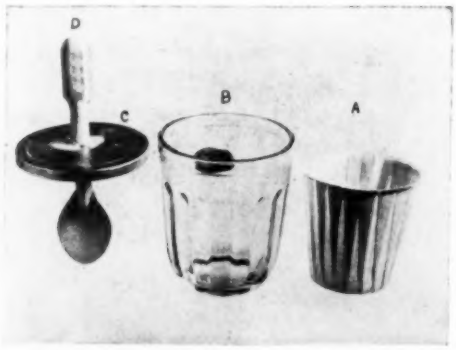

Fig. 1.

container for feces which he has named the "koproscope." It consists essentially of a glass jar with a special screw top, attached to which and projecting into the jar is a metal scoop. The advantages of such a device are obvious. There is described below a somewhat similar contrivance which embodies the principle of the "koproscope," with certain modifications.

In Fig. 1 are shown the component parts of the utensil. *A* is a waxed paper drinking cup of 140 c.c. capacity. *B* is a 210 c.c. glass "jelly jar." *C* is the tin lid of the latter and through which a paper picnic spoon (*D*) has been passed.

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[1]Einhorn, M.: Jour. Am. Med. Assn., 1927, lxxxviii, 1145.

Fig. 2 shows the parts assembled ready for use. The paper cup has been set within the glass jar; the lid, with the spoon, has been placed in position. To utilize the container, the patient or attendant removes the cover, secures a spoonful of fecal material and replaces the lid, leaving the spoon in situ.

To prevent the escape of any obnoxious odors, the small opening in the lid surrounding the handle of the spoon may be closed by means of a piece of adhesive plaster. Also, to guard against soiling the under surface of the cover, a perforated circular piece of waxed paper of the same size as the lid may be placed beneath the latter, prior to collecting the sample.

After the examination of the specimen has been completed, the cup and spoon are discarded and replaced by new ones. Thus no unpleasant cleansing of soiled vessels is necessary.

Fig. 2.

It is true that the specimen cannot be viewed without removing the lid of the container, as it can in the "koproscope," but this is not considered a very serious disadvantage in that inspection of the material is limited to the laboratory worker at the time when the other tests are carried out, and the jar does not need to be wrapped in order to prevent its contents from being exposed to general view.

The container described in this paper has been found to provide a simple, sanitary, convenient, and inexpensive utensil, free from error-producing influences, for obtaining samples of feces as required in routine clinical practice.

The only reason for adopting the particular style of jar as shown in the photographs is that no other glassware of exactly similar pattern is in use in this hospital.

A CLINICAL TEST FOR URINARY ACETONE AND DIACETIC ACID*

By Jeanette Allen Behre, Ph.D., Cincinnati, Ohio

DISCUSSION OF QUALITATIVE TESTS

THE various so-called "tests" for acetone and diacetic acid described in the textbooks and laboratory manuals are open to criticism from at least two points of view when an attempt is made to apply them to routine urinary work. In the first place they cannot be relied upon to give satisfactory results when applied to the urine directly because of the possible presence of interfering substances, and in the second place, in each one of the tests a more intense reaction is given by one of the acetone bodies than by the other, so that it is difficult to assign any definite points at which the test may be called "positive" or "negative." In addition, some of the tests, such as the ferric chloride test for diacetic acid, are far from being sensitive enough to indicate the presence of small amounts of the substance, just above the normal, which may be very significant (1 or 2 mg. per 100 c.c. of urine may be regarded as above the normal).

The *ferric chloride* and *iodoform* tests are both impractical for routine use, the first because it is neither sufficiently specific nor sufficiently sensitive, and the latter because even in its modified forms it is not sufficiently specific to give significant results when carried out on the urine directly.

The *sodium nitroprusside* test, which is one of the most commonly employed, is extremely sensitive to diacetic acid, when carried out with ammonia and an ammonium salt (Rothera's test), but reacts much more slowly and less intensely with acetone. In general, it may be said that diacetic acid reacts quickly in this test, giving a deep rose to permanganate color, and that acetone gives a less intense color, which develops more slowly, but the two types of reaction grade into each other with varying concentrations of the two substances, so that it is impossible to distinguish between moderately large, and quite significant, amounts of acetone, and very small amounts of diacetic acid. Bigwood and Ladd[1] found that diacetic acid showed a positive reaction to a dilution of 1 to 20,000, but that acetone did not give color at a dilution greater than 1 to 1,000. In Cole's *Practical Physiological Chemistry*,[2] it is stated that with Rothera's test acetone can just be detected in a dilution of 1 in 20,000, while aceto-acetic acid shows a positive reaction in a 1 to 400,000 dilution. By a modification of the method, using relatively large amounts of solid ammonium chloride (about 1.5 gm.) with only about 10 drops of the solution to be tested, 1 drop of 5 per cent sodium nitroprusside and a layer of concentrated ammonium hydroxide, we have been able to detect acetone in a 0.002 per cent pure water solution, and diacetic acid at a much greater dilution. In urine, however, the faint pink color given by these small

*From the Biochemical Laboratory of the Union Central Life Insurance Co.
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amounts is usually hidden by the color of the urine itself, or the color developed from other substances. In most specimens of urine the amount of diacetic acid which yields 0.005 per cent of acetone on distillation can be easily detected, but the presence of acetone is often doubtful in the test in concentrations even higher than 0.005 per cent. The nitroprusside test is also far from specific for the acetone bodies. It is used for the detection of glutathione (Hopkins[3]), and in general for the presence of the SH group (Heffter[4] and Arnold[5]). Specimens of urine are often found which give suspicious looking colors not due to acetone bodies. Alkaline urine and urine high in urates often show such color. Formaldehyde in large amounts, and some other substances used as preservatives, give interfering colors.

The *salicylic aldehyde* reaction, carried out in strongly alkaline solution (Frommer's test), is extremely delicate for the detection of acetone but gives very slight color with diacetic acid. It has been found very satisfactory for use on distillates from urine or blood filtrates[6] but cannot be used to advantage on urine (or blood filtrates) direct. This is due to the interference from sugar, which gives color under these conditions, and other substances, some of which tend to inhibit the formation of color. The fact that diacetic acid reacts to a very slight extent but not markedly is also a disadvantage.

As no test was found which gave satisfactory results when used directly on the urine, and as a distillation of all samples is impossible when large numbers are to be tested daily, the following method was devised, using the salicylic aldehyde reaction, in an attempt to obviate these difficulties.

THE CLINICAL TEST

The reagents used are salicylic aldehyde and a concentrated solution of sodium hydroxide. The urine is heated in a test tube in a boiling water-bath and as the vaporized acetone rises, it is brought into contact with the reagents which are suspended over the mouth of the tube on a piece of cotton.

It is best to use a rather wide and short test tube (about 2 cm. in width and 12 cm. in length), but the exact dimensions are not essential. Three c.c. of urine are placed in the clean test tube and one drop of 50 per cent concentrated sulphuric acid is added to facilitate the transformation of diacetic acid to acetone. A small, thin square of cotton, of good quality, is prepared, and one drop of pure, undissolved salicylic aldehyde* is dropped upon the center of it. Two drops of 32 per cent sodium hydroxide are then applied over the spot of aldehyde. This is preferable to adding the hydroxide first. The two reagents solidify on the cotton to form a flat, yellow disc. The cotton is then inverted over the mouth of the test tube so that the spot formed by the reagents is turned down toward the urine. The cotton may be pushed down slighly into the tube to keep it in place, but in such a way that the disc formed by the reagents does not touch the sides of the tube. The tube is then placed in a boiling water-bath for eight minutes. It is convenient to have a rack for holding the tubes upright in the bath. In the presence of

*There is a great difference in the intensity of color given in this reaction by different makes of salicylic aldehyde. The product which we have found the most satisfactory is Eimer and Amend's "Acid Salicylous, Synthetic (Salicylic Aldehyde)." This reagent should be kept in a dark, glass-stoppered bottle. It may be added to the cotton from a dark-colored dropping-bottle.

acetone or diacetic acid, which is changed to acetone, a pink to deep rose color is formed with the reagents on the cotton, quite distinct from the yellowish color given by a blank. The pink color given by the acetone deepens for some time after the cotton has been removed from the tube, so that it is best in doubtful cases to allow the color to deepen in the air for ten or fifteen minutes before making a final decision. Urine containing 1 mg. of acetone per 100 c.c. or the equivalent amount of diacetic acid, shows a very distinct positive color after the heating, when 3 c.c. of urine are used. Any interfering substances which would be eliminated by distillation are also eliminated by this method.

It has been found that in some cases dark greenish rings tend to form around the spot made by the reagents. This is less likely to occur if care is taken that the tube used is perfectly clean, that the reagents do not come in contact with the sides of the tube, and that the cotton completely covers the mouth of the tube.

With a little practice, using uniform conditions, it is quite easy to make a roughly quantitative estimate of the amount of acetone present, judging by the depth of color found on the cotton. For an accurate quantitative estimation a distillation may then be carried out.

It may be of interest to add a word on the subject of the interference of formaldehyde in this reaction, since this substance is so often used in one form or another as a urinary preservative. If even small amounts of formaldehyde are present in an acetone solution, the color given with salicylic aldehyde is very appreciably reduced. This effect is also found in distillates from urine containing formaldehyde and is noticeable in the test described above. It has been found that in this test the inhibiting effect of formaldehyde can be somewhat overcome, when acetone is present, by the addition of one drop of 0.2 per cent potassium bichromate to the urine before it is heated. Diacetic acid is more sensitive to the effect of formaldehyde than is acetone and apparently part of it is lost during the heating with formaldehyde and bichromate, so that the full development of color cannot be expected from diacetic acid if formaldehyde is present.

SUMMARY

A simple method for detecting the presence of acetone and diacetic acid in urine is described, utilizing the salicylic aldehyde reaction. A roughly quantitative estimate may be made of the amount of these substances present. A brief discussion of qualitative tests is included.

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[3]Hopkins, F. G.: Biochem. Jour., 1921, xv, 286.
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[6]Behre, J. A., and Benedict, S. R.: Jour. Biol. Chem., 1926, lxx, 487.

A RAPID TECHNIC FOR THE MULTIPLE TYPING OF BLOOD BY THE MACROSCOPIC AGGLUTINATION METHOD*

By Arthur T. Brice, Jr.,† B.A., Florence, S. C.

WHERE a large group of individuals are to be typed by the macroscopic slide agglutination method, or where time is a consideration of any importance, as is always the case in typing to select a donor for transfusion, we have found the simple device to be described of very considerable value.

During the past three and one-half years there have been performed at the Florence Infirmary over 400 blood transfusions, with a percentage of unfavorable reactions to the patient of practically nil. We attribute this long series of uniformly successful transfusions to the proper selection of donors, which has always been made by the macroscopic slide agglutination method of typing. It is our belief that this method in which the mixed corpuscles of the donor in the serum of the recipient and the corpuscles of the recipient in the serum of the donor's blood are oscillated on a slide to impart motion to the corpuscles is a more perfect and reliable anastamosis than the simple incubation of the drops at body temperature for an arbitrarily selected period of time. Our figures for the past fourteen months show that it has been necessary to type an average of a fraction under ten donors in order to secure the proper blood match for each recipient. We believe that this is a large number in comparison to other methods, but that it indicates the greater sensitivity of the macroscopic method and is well worth the trouble in results to the patient. The device illustrated and to be described has been designed primarily for use in this method, but a modification of it might also be convenient in any of the microscopic technics.

As we have never observed an agglutination reaction to take place in the macroscopic technic after the sixth minute, we type all specimens for a full seven minutes before rendering a decision as to their compatibility. If, then, for example, eight donors are to be typed for one recipient by the single slide method, it would take one operator $8 \times 7 = 56$ minutes to do the actual typing for the group. By the use of the illustrated plate the eight can be typed at one time, thus saving a possible $7 \times 7 = 49$ minutes.

The device consists of a piece of plate glass ¼″ thick by 3¾″ wide by 13″ long, with ground edges and fitted with two removable handles consisting of maple cylinders of 2″ diameter, 3¾″ long, slotted to receive the ends of the glass plate to a snug fit. Between the handles the plate is divided laterally into twelve divisions each 1″ wide by lines ruled on the surface of the glass with a black bitulithic paint, of sufficient thickness to make an embossed ridge on the glass that will prevent the drops of blood from merging into each other when the plate is oscillated. The plate is again divided by two longitudinal lines separating each 1″ division into three parts, in the upper of which being 1″ by ¾″ is ground a ½″ circle on which the laboratory number or designating initial of each specimen may be written, and in each of the two lower subdivisions of which being 1″ by 1½″ a drop

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†Laboratory Director of the Florence Infirmary.

of blood, *patient's cells plus donor's serum* or *patient's serum plus donor's cells,* may be mixed.

It is our custom always to make a graphic diagram of the position on the plate in which each drop of blood is to be mixed, which eliminates all possibility of error and also gives a permanent record that will be of value should it ever be desirable to make a large grouping of the individuals whom we have typed under either of the two generally accepted classification systems. The blood specimens having been taken, defibrinated, and separated by centrifugation we next mix one drop of the recipient's corpuscles with four drops of the serum of each of the donors, and a drop of each of the donor's corpuscles with four drops of the serum of the recipient, with a glass stirring rod, each in its proper place on the typing plate. The plate is then easily lifted from the desk by the cylindrical handles and oscillated with a rocking motion which keeps all of the corpuscles in movement for the desired length of time. We hold the plate while oscillating over a white background, or better still

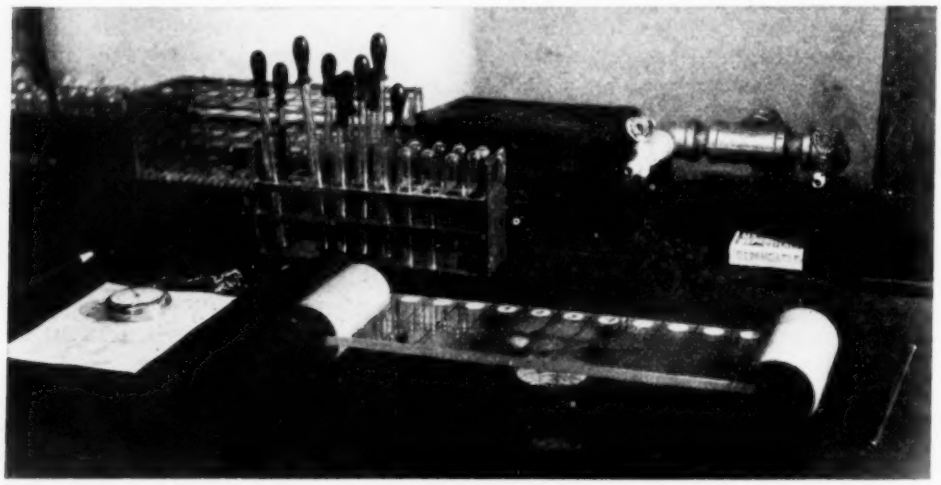

Fig. 1.—Author's blood-typing plate as made for him by the Arthur H. Thomas Company, and a corner of the serologic desk at the Laboratory of the Florence Infirmary.

over a hole in the desk fitted with an electric bulb beneath as used for the microscopes, observing the drops by transmitted light for evidences of agglutination. As agglutination shows up, the donors in question may be ruled out by their number or designating initials, and the selection made at the end of the seven-minute period from among those donors whose specimens have shown no agglutination in either side of the cross-match.

SUMMARY

1. The macroscopic slide agglutination method of blood typing is considered the most sensitive and efficient method for the selection of donors for transfusions.

2. A rapid technic is described making use of a ruled glass plate by means of which a number of donors may be typed against a prospective recipient at one time.

REFERENCE

McLeod, James: The Transfusion of Blood, Jour. S. Carolina Med. Assn., 1926, xxii, 57.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

Live Fishes Impacted in Food and Air Passages of Man, Gudger, E. W. Arch. Path. and Lab. Med., September, 1926, ii, 355.

The data given show that on the coasts of France and Italy and in India and the Far East, fishermen are accustomed to take live fishes between their teeth either to kill them by biting, or to hold them while the hands are used to free the hook or net. A fish so held, if it pricks the man's mouth with its spines or sets up any sudden movement, will readily cause the involuntary opening of the mouth with a resultant entrance of the fish into the throat of the man.

In time, accounts of the impaction of live fishes in the food and air passages range from Cognatus, 1567, to Jordan, 1924—a range of 357 years.

Twelve countries are represented in these various accounts as follows: India leads with five cases, and France follows with three; Burma and Italy tie for third place with two cases each; Algeria, Ceylon, Germany, Japan, Philippine Islands, Scotland, and Senegal are credited with one each; and finally there is one account (Cognatus', the oldest) not assignable to any country.

ARTHRITIS DEFORMANS: The Rôle of the Dysentery Bacillus in Arthritis Deformans, Clifford, S. H. Am. Jour. Dis. Child., July, 1926, xxxii, 72.

That the symptom complex called arthritis deformans of Type I may, in some cases, be the arthritis following an unrecognized dysentery bacillus infection is the possibility raised by the observations here reported. Seven cases of arthritis deformans are reported in four of which there was evidence of either an active or a past infection with B. dysenteriae, Flexner.

The literature shows arthritis following a typical dysentery to be a well-established clinical entity that may occur as long as four weeks after the acute attack.

It is possible to have a dysentery bacillus infection without its producing a clinical syndrome recognizable as dysentery.

Dysentery should be considered as an etiologic factor in arthritis deformans (Type I), and only ruled out after bacteriologic studies and agglutination reactions, using a complete series of antigens, are proved to be negative.

Under the exceptional conditions governing these observations, the dysenterin reaction proved to be a specific diagnostic test. It has not been studied on a large enough scale to permit any general conclusions as to its value.

The term "dysenterin test" is applied to the intradermic injection of 0.1 c.c. of formalinized dysentry vaccine.

Xanthochromia of the Spinal Fluid in the Newborn, Kohlbry, O. Am. Jour. Dis. Child., July, 1926, xxxii, 58.

A series of five newborn infants with xanthochromia of the spinal fluid is here presented.

The color of the fluid varied from a pale yellow to a bright canary. All these fluids showed red blood cells, either all or in part crenated, when examined a few minutes after withdrawal. No difficulty was encountered with any of the spinal punctures. They were what might be termed "clean punctures" in that there was no evidence of macroscopic

blood at the time. Some signs of intracranial disturbance were noticed before punctures were done in all these cases. These signs, in the order of their frequency, were: uncontrollable vomiting, listlessness, mild cyanosis, nystagmus, and a very high temperature.

There is evidence, based on the characteristics of these fluids and the clinical course of these infants, that yellow fluids, such as are here described are related to previous intracranial blood vessel derangement, producing either transudation of blood plasma, frank hemorrhage, or both. The etiologic agent may be intracranial congestion occurring during labor.

The material here presented is purely clinical. Further knowledge of this condition will be gained only as such fluids are examined more carefully, with particular reference to coagulability, albumin content, cell content, and the presence or absence of bile and hemoglobin or their derivatives.

MEASLES PROPHYLAXIS: Report on the Use of Immune Goat Serum, Hayne, A. H., and Gasul, B. M. Jour. Am. Med. Assn., October 16, 1926, lxxxvii, 1185.

Tunnicliff's immune measles serum has been of definite value as a prophylactic for measles.

If the serum is administered to contacts not later than the fifth day of exposure, always counting the date of onset as the first day of the disease, protection seems assured in about 90 per cent.

Those who receive serum early, yet are not wholly protected, pass through an attenuated form of the disease.

Passive immunity endures from two to four weeks and in some instances even longer. A child was placed, through error, in a measles ward two weeks after he had received 7 c.c. of immune goat serum intramuscularly. This child, because of his close contact with many cases of measles in all stages of the disease, was believed to be hopelessly exposed and so was not removed from the ward, where he remained for more than two weeks. He did not contract measles.

The serum produced no deleterious effects, regardless of age or physical condition of the patients in whom it was used.

The advantages of the Tunnicliff serum, as compared with convalescent measles serum, are evident when the question of supply is considered. Ultimately, it is hoped, the Tunnicliff serum will be easily available for all who desire it, whereas a similar situation can scarcely be hoped for in respect to convalescent serum.

LIVER DAMAGE IN TREATED SYPHILIS: Urobilinogen Determination in Treated Syphilitic Patients, Brown, H., and Greenbaum, S. S. Arch. Dermat. and Syph., October, 1926, xiv, 434.

A sensitive test of liver function is of prime importance to the syphilographer because of the well known effects on the liver of commonly-used antisyphilitic remedies.

The inability of damaged liver cells to remove urobilin from the portal circulation necessitates elimination by the kidneys, and therefore it has been proposed that some idea as to the presence or even the extent of hepatic damage may be had from examinations for urobilinuria.

The authors studied the question by the application of the test described by Wallace and Diamond (*Arch. Int. Med.*, June, 1925, xxxv, 698) to 100 cases of syphilis under treatment. Over 500 determinations were made. Their conclusions were that the results of urobilinogen determinations on the urine of treated syphilities were too variable to serve as an index of hepatic damage in such patients.

BLOOD SEDIMENTATION: Significance of Blood Sedimentation in Urology, Litten, L., and Szpiro, J. Ztschr. f. Urol., 1926, xx, 481.

The authors made 118 tests on 41 patients in the Urologic Section of their hospital by Linzenmeier's method and came to the following conclusions: The value of the sedimentation test in differential diagnosis between inflammatory and noninflammatory diseases and between

benign and malignant newgrowths was confirmed, but it must always be interpreted in connection with the other clinical findings. With this reservation the sedimentation test is valuable in urology in distinguishing between hypertrophy and cancer of the prostate and in the differential diagnosis of kidney colic. Litten's curve is recommended in the differentiation of the course of sedimentation. This curve shows that where there is greatly increased rapidity of sedimentation the severity of the pathologic process is shown in a particularly rapid fall in the terminal sedimentation. Sometimes in doubtful cases the sedimentation test can be judged only by the use of these curves. By repeated reading of the sedimentation time at certain intervals and by the course of the sedimentation, a true picture of the course of the disease can be obtained and a more accurate prognosis can be reached than by other methods.

KIDNEY FUNCTION: The Rate of Filtration and Reabsorption in the Human Kidneys, Rehberg, P. B. Biochem. Jour., London, 1926, xx, 447.

The filtration-reabsorption theory is discussed. Its present state calls for a thorough modification, or for the complete abandonment of filtration as the main factor in excretion by the kidney.

The amount of creatinine present in the urine of man after ingestion of this substance is so large that it requires a filtration of up to 200 c.c. per min. to explain it.

The possibility of this is discussed and the result is taken to be in favor of the filtration theory.

The surface available for reabsorption in the proximal convoluted tubes alone is so large that, provided it shows only the same power of reabsorption as the cloaca of the bird, it may reabsorb the whole quantity of fluid required in the concentration process in the tubules.

The different ways in which a diuresis may be obtained according to the filtration-reabsorption theory are enumerated and discussed with examples.

BISMUTH, EFFECT OF ON KIDNEY: A Study of the Blood Chemistry and the Histopathology of the Kidneys After Experimental Bismuth Injections, Brown, H., Saleeby, E. R., and Schamberg, J. F. Jour. Pharmacol. and Exper. Therap., August, 1926, xxviii, 165.

These investigations were prompted by the increasing use of bismuth therapy in syphilis. A single product—potassium tartrobismuthate in olive oil—was used for experiments on rabbits from which it was found that:

The histopathologic changes in the kidney sections correspond in general with the blood urea findings. The changes in one rabbit were severe; in another less severe, and in another the least changes were noted. It would appear, therefore, that blood chemistry tests would prove of value in controlling bismuth therapy in human beings.

These studies indicate that bismuth is a drug of relatively low toxicity. When one considers that as high as 30 mg. per kilo (9 times the therapeutic dose for the human species) were injected into rabbits without producing an alteration in the blood chemistry indicative of nephritis or histologic evidence of material kidney damages, the latitude between the therapeutic and toxic dose may be appreciated.

RETICULOCYTES: The Clinical Significance of the Reticulated Red Cells, Damashek, W. Boston Med. and Surg. Jour., April 29, 1926, cxciv, 759.

Polychromatophilia, a universally conceded sign of regeneration in the fixed preparation runs parallel at times to the reticulation of the unfixed smear.

Aplastic anemia, which is an extremely rare condition, represents a rapid failure in bone marrow growth, with resultant anemia and death. Accordingly, the reticulate count would be expected to be almost nil and to remain so until death. The few cases that were observed bore out this assumption.

In purpura hemorrhagica, in which there is a failure in platelet growth resulting in hemorrhages and anemia, it was observed that when the reticulate count was low and remained so, the patients continued to bleed and finally died; on the other hand, when the reticulate count became high, the platelets increased and the patient recovered.

Marked reticulosis in congenital hemolytic anemia is a pathognomonic point in the field of diagnosis, differentiating that disease from all other anemias with large spleens.

In the anemias of pregnancy, the reticulate count acts as it does in the other anemias. In the leucemias and diseases of lymphoid origin, the reticulate count may be slightly elevated, but without relation to the course or scope of the disease.

APLASTIC ANEMIA: Differentiation of Aplastic Anemia and Essential Thrombocytopenia, McElroy, J. B. South. Med. Jour., May, 1926, xix, 325.

Aplastic anemia is a severe myelopathy with deficient regeneration of the blood elements, characterized in many cases by extreme pallor of the skin, hemorrhages from the mucous membranes, petechiae and ecchymoses, absence of splenic enlargement, diminished hemoglobin metabolism, absence of plastic changes in the red blood cells, leucopenia and relative lymphocytosis, pronounced thrombocytopenia with lack of reaction from adrenalin and splenectomy, Rumpel-Leed's phenomenon, prolonged bleeding time, nonretractility of clot, reduced bilirubinemia, and aplastic bone marrow, which resists all forms of treatment and in which splenectomy is positively contraindicated.

Essential thrombocytopenia is a thrombolytic purpura, differing from the above in periodical occurrences of hemorrhages from the mucous membranes, frequently enlarged spleen, normal hemoglobin metabolism, presence of plastic changes in the red blood cells, increase of blood platelets after adrenalin and splenectomy, and a varigated bone marrow which is often benefited and frequently cured by splenectomy.

	APLASTIC ANEMIA	ESSENTIAL THROMBOCYTOPENIA
I. *General Symptoms:*		
a. color of skin	alabaster	variable
b. skin and mucous membrane hemorrhages	usually pronounced	periodical occurrence with free intervals
c. mouth cavity	frequently gingivitis, stomatitis, angina necrotans	numerous small hemorrhages, almost always gingivitis
d. spleen	often small, not palpable	enlargement may be present
e. urine	no urobilinuria	often urobilinogen
f. gastric secretion	often disturbed	usually normal
g. stool	very slight urobilin content	normal urobilin content
II. *Blood Findings:*		
1. Morphology		
a. erythrocytes	oligocythemia, progressive, poikilocytosis, polychromasia; reticulated cells absent	number variable, often evidence of regeneration
b. leucocytes	leucopenia, marked reduction of granulocytes, relative lymphocytosis	often leucocytosis, differential count shows nothing distinctive
c. blood plates	pronounced thrombopenia, (adrenalin and splenectomy do not produce an increase in thrombocytes)	during attack marked thrombopenia; after adrenalin and splenectomy an increase of platelets
2. Stasis experiment	skin hemorrhages	skin hemorrhages
3. Bleeding time	markedly prolonged	prolonged during attack
4. Retractility of clot	absent	absent
5. Color index	usually one	below one
6. Bilirubin content	reduced	normal

LABORATORY TECHNIC

BILIRUBINEMIA IN PREGNANCY: The Diagnostic Value of Bilirubinemia in Pregnancy, Mandelstamm, A., and Nageikoff, L. Monatschr. f. Geburtsh u. Gynäk., June, 1926, lxxii, 297.

The plasma is separated from citrated blood and 0.5 c.c. placed in a watch glass. One c.c. of 20 per cent trichloracetic acid is added, the mixture stirred with a glass rod and filtered through a small filter.

When the filter paper dries at room temperature, a positive reaction is indicated by a green color. With a negative reaction the sediment remains yellow or dirty white.

The reaction is always negative in normal cases. When used for the diagnosis of pregnancy, all factors leading to increased bilirubin in the blood, such as heart and liver disease, must be excluded.

The authors tested 134 cases and concluded that the test was a frequent but not constant sign of pregnancy. It is of no value in the differentiation of tubal pregnancy and adnexal inflammation as it may be positive in the latter.

HEMOLYSIN FORMATION: The Formation of Antisheep Hemolytic Amboceptor in the Normal and Tuberculous Guinea Pig, Louis, P. A., and Loomis, D. Jour. Exper. Med., October, 1926, xl, 503.

The guinea pig infected with virulent tubercle bacilli develops much more antisheep amboceptor than do controls when given like amounts of sheep red blood corpuscles.

The curve of antibody production in the guinea pig when treated with sheep red blood corpuscles shows a departure from curves previously determined in other animals.

These facts were ascertained as part of an effort to learn more of the functional nature of the inheritable factors controlling natural resistance to disease.

BLOOD SUGAR: A Study of the New Benedict Method for the Determination of Blood Sugar, Rockwood, R. Jour. Biol. Chem., July, 1926, lxix, 187.

The new Benedict method for blood sugar was compared to the Folin-Wu in a series of 300 samples of blood.

The Benedict reagents which have been used up to the present time do not require so great a correction for lack of proportion in color development as the Folin-Wu.

The normal cases in this group show a mode between 75 and 85 mg. per 100 c.c., and a range of from 50 to 120 mg.

The difference value between the Folin-Wu and Benedict methods does not seem to be correlated with any factor in the condition of the diabetic patients.

The cuprous oxide precipitate in the Benedict test is less soluble than in the Folin-Wu, especially with high blood sugars.

The substance causing the difference value is apparently not one of the ordinary nitrogenous reducing substances since the difference value is not increased in uremia.

Sugar-tolerance tests show no characteristic variation in the difference value.

The substance causing the difference value seems to be present in greater quantity in the cells than in the plasma and is sometimes only present there.

SPIROCHETA PALLIDA: Congo Red and Hydrochloric Acid or Nitric Acid (Fuming) Method for Treponema Pallidum Examination, Udasco, L. Jour. Philippine Med. Assn., June, 1926, vi, 196.

"(a) Take a small drop of secretion from a suspected lesion and deposit it on one end of a clean slide. Emulsify it with an equal quantity of Congo Red (2.5 per cent in water). Make a smear as for blood. Air dry.

"(b) Pour enough concentrated HCl or HNO_3 in a small Petri dish, or similar container, and place the slide on the rim of the dish, smeared surface down, so as to come

in contact only with the fumes of the acid. Almost instantly the smear turns blue and in thirty seconds is ready to be examined under oil immersion objective. The Treponema pallidum will be found as clear white spirals against a uniformly blue background."

GLUCOSE ESTIMATION: On the Estimation of Glucose in the Presence of Phosphate Buffers. Visscher, M. B. Jour. Biol. Chem., July, 1926, lxix, 9.

It is pointed out that the presence of potassium acid phosphate in glucose solutions lowers the reducing power of the glucose for copper. This fact must be taken into account whenever the sugar content of buffered solutions is measured.

SYPHILIS, FLOCCULATION TEST WITH DRIED FISH EXTRACT: Chemical Application of Flocculation Reaction With Katsuobushi (Dried Japanese Fish) With Blood Serum and Plasma in Syphilitic Children, Takeda, S. Oriental Jour. Dis. Infants, July, 1926, i, 81.

The author cut up Katsuobushi, mixed it with ether, and let it remain for three days at room temperature (15° C.), changing the ether several times. After removing the ether, he added 85 per cent alcohol in proportion of 1:5, after which the mixture was allowed to stand for one week at room temperature and then filtered.

The test gives results approximating those of the Wassermann in 85 per cent of 160 cases. This test was also tried in 400 cases by Sasaki (Sasaki, R: *Oriental Jour. Dis. Infants*, July, 1926, i, 82) with an agreement of 89 to 90 per cent in activated or inactivated sera and an agreement with Wassermann reaction of 88.75 per cent.

DIPHTHERIA BACILLUS: The Corynebacteria (Diphtheria Bacillus and "Diphtheroids"). The Importance of Microphotography as an Aid to Their Classification and Identification, Thomson, D., and Thomson, R. Annals Pickett-Thomson Res. Lab., London, 1926, ii, 51.

The authors comment upon the fact that there are hundreds rather than a score of "diphtheroids" whose satisfactory classification is a matter of great confusion and difficulty, which has been farther intensified by the habit of applying definite names to diphtheroids which have been inadequately described.

They contend that the clearest, most easily and rapidly comprehended, as well as the best method of recording the diphtheroids in an orderly and accurate manner is to give a careful and complete list of tests and characteristics of each, accompanied by as many good photographs as possible.

In pursuance of this plan, in a paper covering 149 pages, they present such a study of the diphtheria and "diphtheroid" strains (72) embodying complete cultural and microphotographic characteristics.

The paper is illustrated with 130 microphotographs which well repay study.

Their study involves routine culture on the testicular infusion-peptone-glucose agar (*Annals Pickett-Thomson Res. Lab.*, i, 223) and on the following sugars: glucose, maltose, galactose, saccharose, lactose, mannite, and dextrin. Frequent microphotographs of both Gram-stained specimens and growth in culture form the remainder of their basis for classification.

The paper contains a wealth of data and can be studied with profit.

PRECIPITATION TEST FOR SYPHILIS: A Simple Rapid Precipitation Test for the Diagnosis of Syphilis, Butler, H. W. New Orleans Med. and Surg. Jour., August, 1926, lxxix, 105.

Antigen Preparation.—Fresh baby veal hearts are selected, and the superficial fat is removed. The muscle is ground in a sausage grinder, spread on paper and dried with the aid of an electric fan. After it is completely dried, it is powdered in a mortar and extracted with ether. Four hundred c.c. of ether are used to each 100 gm. of powdered heart. This is allowed to act for ten minutes, shaking frequently, and the ether is then

filtered off. The material is extracted again three different times with 300 c.c. of ether, which is filtered off and discarded in each instance. The ether-extracted powdered heart muscle is now dried free from ether, and for each gram of the dry material, 5 c.c. of 95 per cent alcohol are added and maceration is allowed to continue for three days at room temperature, after which the alcohol is filtered off and enough 95 per cent alcohol added to bring it up to the original volume. This constitutes the defatted alcoholic heart extract for the antigen. Six decigrams of cholesterol and 3 c.c. of glacial acetic acid are added for each 100 c.c. of the alcoholic extract. This is filtered after solution is effected and constitutes the finished special antigen for this test. This antigen seems to be stable for at least several weeks.

Technic.—One c.c. of antigen is measured into a test tube and 2 c.c. of distilled water into a second tube (normal saline can be used, but the solution is unstable). Mixing is effected by pouring the solution from one tube to the other, back and forth for at least six times.

Two drops of serum are placed upon a clean slide about the junction of the middle and outer third. With a pipette, used only for the antigen dilution, three drops of the dilution are placed upon the slide near, but not into, the serum. These are mixed on the slide thoroughly with the end of a toothpick or other suitable instrument, and the slide is slowly rocked for two minutes. If the serum is positive, a characteristic granular precipitate which can be easily seen, develops during the rocking process. If negative, no specific precipitate forms within the two minute time limit.

SPIROCHETA PALLIDA, STAINING OF: Staining Spirocheta Pallida in Sections by Silver Impregnation, Lukes, J., and Jelinek, V. Ztschr. f. Immunol. u. Exper. Therap., April 8, 1926, xlvii, 83.

The authors have had good results with the following method:

Formol fixation. Alcohol twenty-four hours. Place in water until the pieces fall to the bottom. Silver nitrate 2 per cent for two or three days. Short irrigation with distilled water. Thirty per cent grape-sugar solution for two days. Place in water for "a long time." Frozen sections or embedding.

They also used, with success, the solution below instead of glucose as an oxidizing agent:

Onion skins are macerated and boiled for a long time; then they are pressed and filtered several times until the fluid becomes clear. This is allowed to ferment and is then sterilized and filtered.

If a rapid method is desired, sections that have been well dehydrated in alcohol (up to two hours) are put in distilled water and left until they fall to the bottom. They are then put in 2 or 3 per cent silver nitrate for three hours at 56° and then washed in water and reduced in onion stain for two or three hours at the same temperature. The sections are then washed with alcohol; balsam.

ANEROBES: A New Method for the Cultivation of Anerobic Bacteria With Cystein in Media, Hosoya, S. Japan Med. World, April 15, 1926, vi, 83.

Broth containing 0.001 per cent of l-cystein hydrochloride adjusted to P_H 7.2 to 7.1 gives a profuse and rapid growth of obligate anerobes without a vaseline seal.

OVA IN FECES: A Method for the Routine Examination of Feces for the Ova of Parasitic Worms and Encysted Amebas, Johns, F. M. New Orleans Med. and Surg. Jour., 1926, lxxix, 218.

Formed or semisolid stools are diluted from 10 to 15 times with water, shaken well, and strained through two layers of cheesecloth directly into a 12 by 115 mm. (½ by 4 inch) *round bottom* tube. Balance tube in centrifuge. (This should be equipped with Cornell shields and should be able to run at least 1500 r.p.m.) Start centrifuge and immediately advance to full speed of 1500 r.p.m. Shut off power within from fifteen to twenty seconds after

advancing the speed. Pour off the supernatant fluid, resuspend sediment with water and re-centrifuge. (Another such washing will frequently clear the sediment still more.) Decant again, leaving a few drops of water with which to shake up the sediment. Pour on slide and spread out to where all particles on the slide are easily visible under the low dry lens and examine as for parasite ova.

DUCREY BACILLUS: Technic of the Isolation of the Streptobacillus of Ducrey, Durand, P. Compt. rend. Soc. de biol., Paris, June 4, 1926, xciv, 1324.

Media.—1. Agar with sheep's blood. One hundred and fifty c.c. of ordinary agar (3 per cent) in peptonized beef bouillon of about P_H 7.6 previously sterilized in an Erlenmeyer flask with a capacity of 250 c.c. is placed over a water-bath until completely melted. This nutritive medium is then cooled to about 45° C. and from 30 to 50 c.c. of fresh defibrinated sheep's blood is added. Sheep's blood which has been kept aseptically may be used. The medium is divided into inclined tubes which may be used in a few hours or as long as they are not too dry.

2. Pure sheep's blood. Sheep's blood obtained aseptically from the juglar vein is immediately divided into tubes 11 cm. by 11 mm. which are placed in an inclined position to favor coagulation. At the end of a few hours they are placed in an upright position, and the serum soon separates from the clot.

These tubes may be used as soon as the serum exudes and separates from the clot, but it is better to wait for half a day. They remain good as long as they have not dried out, even if the serum is tinted with hemoglobin. Both media should be stoppered and kept in an ice box if they are not to be used for some time.

If the bubo is not opened, puncture should be made with a large needle on a tight syringe. If the bubo is open, paint it with tincture of iodine after slight pressure, then apply a tampon wet with alcohol. The sample is taken from the interior with a pipette. In the case of chancres, if possible, select one in the state of a pustule which has not yet opened. Touch with tincture of iodine, then with alcohol. Remove the epidermal layer aseptically and obtain the pus with a platinum wire or a pipette. When the chancres are wide open, the whole surface must be sterilized and the streptobacillus obtained from the deeper layers. In order to do this, clean carefully with gauze, then apply a tampon of tincture of iodine for three or four seconds, wash rapidly with alcohol, and dry with gauze. The chancre is then squeezed with the fingers until a serous fluid exudes. This fluid, which may be blood-tinged, is picked up with a pipette.

The agar with sheep's blood is an excellent medium for the bacillus of Ducrey, but it is also favorable for many saphrophytes, hence this medium is used for clean products (unopened buboes or chancres). Less material is required and more tubes are to be used, and the material is to be spread over the whole surface of the tube when the lesions are open.

The tubes of pure blood are less favorable for an abundant development of the streptobacillus, but it is less favorable for other bacilli. The material should be implanted in the serum, not in the clot.

The tubes are placed in an incubator with a rather low temperature, preferably 30° to 36° C. On blood agar typical colonies of the Ducrey bacillus develop in from thirty-six to forty-eight hours. On pure sheep's blood the growth should be looked for in stained preparations. The bacilli are very few in number, and they often show involution forms. As soon as they appear, they should be transplanted on tubes of blood agar.

Positive results were obtained in 39 out of 40 cases by these methods.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

New Books Offered to Profession

THE new Illustrated Catalogue just issued by W. B. Saunders Company, medical publishers of Philadelphia and London, describes and illustrates more than 250 titles. Of these, many are important new books or new editions not described in the former issue of their Catalogue.

A serviceable feature of Saunders' Catalogue is the giving of the month and year of publication of each book listed. This, together with the description and in many cases the table of contents, author, and price, makes the Saunders Catalogue one of unusual accuracy and precision from which the doctor may select and order his medical books.

A number of new books in this Catalogue deserve the particular attention of progressive physicians and surgeons. For instance, there are Blumer's Bedside Diagnosis, McLester's Nutrition and Diet, Braasch's Urography, Cecil's new Textbook of Medicine, Stokes' Clinical Syphilology, Kolmer's Chemotherapy, Ford's Bacteriology, Young's Urology, Rehfuss' Diseases of the Stomach, Pelouze's Gonorrhea in the Male, Crohn's Affections of the Stomach, Ewing's Neoplastic Diseases, de Takats' Local Anesthesia, Wechsler's Clinical Neurology, Palfrey's Specialties in General Medicine, etc., etc. Anyone desiring a copy of this 80-page Catalogue needs but indicate his wish to Saunders Company and one will be sent him immediately. It is worth having in the library.

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*Standard Methods of the New York State Board of Health**

IN THIS volume are presented in detail the methods used in the various departments of the New York State Board of Health.

The volume is divided into the sections listed below:

I. *General Laboratory Procedures,* in which the handling of specimens, the preparation and use of stains, and the handling of experimental and test animals are succinctly but clearly outlined.

II. *The Preparation of Media and Glassware.*—In this section the directions are precise and somewhat elementary, as they are intended for workers, as a rule, unfamiliar with laboratory regulations. A variety of media are described, and this section alone will be a useful source of reference in laboratories in general.

III. *Methods Used in the General Diagnostic Laboratories.*—This section, while intended for those having some experience in laboratory technic, is nevertheless complete and not lacking in detail.

One regrets that, in view of the purpose of the book, Orskov's method of single-cell culture is not described but merely referred to as reliable and simple; nor are there detailed directions for micrometry or dark-field examinations. One notes also that hemoglobin is reported in per cent, in spite of the inaccuracy this involves.

*Standard Methods of the Division of Laboratories and Research of the New York Board of Health. By A. B. Wadsworth, M.D. Cloth. Pp. 704. 56 Illustrations, 12 plates. Williams and Wilkins Co., Baltimore.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

IV. *Methods Used in the Laboratories for Sanitary and Analytic Chemistry,* in which are described the examination of water, sewage, effluents, wastes, ice, milk, and cream.

V. *Methods Used in the Antitoxin, Serum and Vaccine Laboratories:* In view of the empiricism of laboratory practice in this field, this section will be of great value and will be read with great interest by those engaged in this work. In view, also, of the empiricism not uncommonly encountered in the use of such agents the section could perhaps also be read with profit by many practicing physicians. This is the longest section of the book.

The last two sections are concerned with the methods used in the executive, research, publication, and library departments.

All in all the book is a valuable and noteworthy publication and typographically exemplifies the motto of the publisher.

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*The Tongue and Its Diseases**

AN ENGLISH surgeon covers the tongue and the diseases to which it is heir in textbook proportions. There is probably no part of the body about which the average practitioner of medicine knows less. Although written from the surgeon's viewpoint, the internist will find this volume a very useful addition to his reference library.

"Perhaps the chief lesson of this book is that cancer of the tongue, in place of being the hopeless disease that the laity and many physicians and some surgeons seem to think it is, is just as curable in its early stages as is cancer elsewhere."

The volume contains a wealth of interesting, as well as useful, illustrations.

The Ear, Nose, and Throat in General Practice†

AS IN other works of its kind, the author, a specialist, frequently overshoots the bounds of general practice. It is in no sense a textbook, or one for reference. After perusing its pages one feels as if he has been listening to fatherly advice. There are excellent chapters on the general practitioner's opportunities and responsibilities in acute ear disease. The nose is probably not so well done. Foreign body in the food and air passages is touched on as well as the technic of tracheotomy and diagnosis of acute and chronic sinusitis. Tonsils and adenoids are not overlooked, in fact the former is overdone from the practitioner's viewpoint. Space is judiciously apportioned to the more uncommon conditions.

The author's plea for early diagnosis and prompt action in acute ear disease is earnest and convincing.

The fact that many common diseases of the ear, nose, and throat are preventable places a great responsibility on the family doctor. In the direction of the life of a patient, the specialist is practically helpless. It is the general practitioner who must impress upon his patient the blessed value of "facing up to" the weather, apply silver nitrate in his first attack of tonsillitis, or apprehensively see him with his first earache.

The Diagnosis of Pancreatic Disease‡

A BRITISH chemico-clinician's views on the manifestations of pancreatic pathology rather tersely and intelligibly stated. The work presents an anatomic, physiologic, and pathologic background, which although not treated in an exhaustive manner, furnishes satisfactory

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*The Tongue and Its Diseases. By Duncan C. L. Fitzwilliams, C.M.G., M.D., Ch.M., F.R.C.S. Edin. and Eng., Surgeon and Lecturer on Surgery to St. Mary's Hospital, Paddington Green Children's Hospital and Mount Vernon Hospital. Cloth. Illustrated. Pp. 505. Oxford University Press. American Branch, New York, 1927.

†The Ear, Nose, and Throat in General Practice. An Informal Guide to the Main Principles. By D. A. Crow, M.B., Ch.B. (Edin.) Oto-Laryngologist, The Royal Sussex County Hospital, Brighton, The Sussex Throat and Ear Hospital, Etc. Illustrated. Cloth. Pp. 150. Oxford University Press. American Branch, New York, 1927.

‡The Diagnosis of Pancreatic Disease. By Robert Coope, M.D., B.Sc., M.R.C.P. Senior Assistant Physician, Royal Southern Hospital, Liverpool; Assistant Physician, Hospital for Consumption and Disease of the Chest, Liverpool; Lecturer in Clinical Chemistry, University of Liverpool. Cloth. Pp. 112. Oxford University Press. American Branch, New York.

orientation. Of course a classification of pancreatic lesions is included. Acute pancreatitis is covered expeditiously in order that the main theme, chronic pancreatitis, may be arrived at logically. The latter is rather comprehensively treated under the following headings: clinical observations including x-ray findings; attempts to detect failure of the discharge of pancreatic juice into the duodenum; attempts to detect failure of insulin production; the findings and opinion of a surgeon during an abdominal operation; the therapeutic test, and esoteric tests with dubious foundations. About one hundred and seventy references are included.

The author is somewhat dogmatic at times, criticizing and denouncing diagnostic methods which he admits never having tried. He believes the most important test of chronic pancreatic dysfunction to be the finding of an abundance of striated muscle fibers in the feces of persons receiving an optimum of meat in their diets. This is very encouraging for the busy internist or surgeon who becomes lost in a maze of complicating technics.

On the whole it is a very delightful essay on the "Cinderella of the Abdomen."

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*The Internal Secretions of the Sex Glands**

NOT a series of popular exaggerations and unwarranted theories but a critical review of the scientific literature on the subject into which is incorporated the author's own experiments, experiences, and interpretations. His references to the literature are most extensive and include work done and observations made not only on man but on a wide variety of animals, such as dogs, fowl, fish, amphibians, and insects. The work might be characterized as a study of the comparative physiology and pathology of the internal secretions of the sex glands. It will have its largest audience among the endocrinologists.

The author presents a summary of the literature dealing with the chemical aspects of internal secretions. Having an interest in the subject, the authors were impressed by the absence of any collective account of the original contributions on the subject and this they present in the work under review. In it will be found a description of the history, methods of preparation, and chemical and physiologic properties of the various internal secretions.

The Transfusion of Blood†

IT HAS been said that the first transfusion was performed in 1492 by a Jewish physician who gave blood to Pope Innocent VIII. According to the story the blood was obtained by bleeding three boys to death. This is probably fiction, since knowledge that the blood circulates dates only from 1628. In the Middle Ages the value of blood as a health restorative was recognized, but during these times it was taken by mouth. The first successful transfusion of blood in animals was performed in 1665; the first in man was in 1667. In this case the blood of a lamb was injected into the veins of a boy, who curiously enough, recovered. Animals were often used thereafter with many consequent deaths, so that after a time blood transfusion was forbidden in France. Even after a reasonably satisfactory method of blood transfer from man to man had been developed (1835), many deaths resulted, and it was not until 1907, when we learned that there are at least four groups or types of blood which can be readily identified and some of which are incompatible with each other, that transfusion was placed on a firm basis. Since then preliminary blood matching has practically obviated serious transfusion reactions. The more extensive utilization of this therapeutic measure has since depended upon improvements in operative methods. The earlier arrangements were crude indeed when compared with the present day technic, and attempts

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*The Internal Secretions of the Sex Glands. The Problem of the "Puberty Gland." By Alexander Lipschutz, M.D., Professor of Physiology in Dorpat University (Estonia), formerly Privatdocent of Physiology in Berne University (Switzerland). With a Preface by F. H. A. Marshall, F.R.S., Author of *The Physiology of Reproduction*. With over 140 illustrations in the text. Cloth. Pp. 513. Williams & Wilkins Co., Baltimore, Md.

†Transfusion of Blood. By Henry M. Feinblatt. Cloth. Pp. 176. MacMillan Co.

at transfusion even in expert hands were often unsuccessful. This was chiefly due to the tendency of the blood to clot before completion of the transfusion.

In 1914 anticoagulants were first satisfactorily used in clinical work so that blood could be drawn into receptacles where it would not clot. With this method the donor and the recipient need not be in the same room, nor indeed in the same building. The vast number of these so-called indirect transfusions during the World War indicated convincingly the great value of blood administration in certain conditions, and this experience went far in popularizing the procedure.

However, the generally accepted opinion has been that whole blood is more effective than blood which has been treated to prevent coagulation, and within the last few years instruments have been devised which have so simplified the direct method that it has come into quite general use in those cases in which transfusion is indicated. The present day procedure possesses the additional advantage that it is not necessary to cut down upon veins and arteries thereby destroying them for future use. So satisfactory are these methods that one group of authors was able to report in 1926 the carrying out of 1000 transfusions all of which were successful as far as the transfusion was concerned.

Blood transfusion is often indicated in a wide variety of conditions, such as sudden loss of blood from any cause, surgical shock, illuminating gas poisoning, chronic hemorrhagic diseases, as a preoperative precaution when the bleeding and clotting time of the patient's blood has been found to be delayed, in bacterial infection, debilitating conditions, diseases associated with blood destruction, and the like.

Dr. Feinblatt contributes a valuable monograph on blood transfusion in which after an historical review and a study of the various methods, he describes in detail his own method, which may certainly be recommended for simplicity and efficiency.

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*Technic in the Management of Diabetic Patients**

ACUPUNCTURE has become such a prominent part of the medicine of today and the home administration of hyperdermics has become so widespread since the advent of insulin that haphazard technic, unnecessary jabbing, and the use of crude instruments, such as improperly sharpened needles, is readily detected by the observant patient.

Skill in this line is chiefly a matter of experience and repetition, but there are many little tricks which will promote success and which the author describes in his small brochure. No technician or physician will read it without getting at least one or two little points which he had not previously realized. The author also incorporates chapters on the preparation and intravenous administration of glucose, transportation of blood, and a practical outfit for urinalysis.

Gastric Function in Health and Disease†

RYLE believes that while hunger is associated with peristaltic contraction of the stomach wall, appetite depends upon the degree of its muscular tone. It is the flaccid, atonic stomach that is associated with anorexia, while the tonic or hypertonic organ is usually accompanied by good or abnormal appetite. Appetite is a function of muscular tonicity. Hunger is a function of muscular contraction.

An analogy exists in the skeletal muscles. Good muscular tone gives its possessor a sensation of well being and an inclination to active movement. Actual contraction of the muscles results in the movement.

Recent work would indicate that the level of gastric acidity is not maintained so much by the amount of secretion of hydrochloric acid as it is by the extent of alkaline regurgita-

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*Technic in the Management of Diabetic Patients. By Henry J. John, M.A., M.D. Cloth. Illustrated. Pp. 62. The Williams Feather Company, Cleveland, Ohio, 1927.

†Gastric Function in Health and Disease. By John A. Ryle, M.D., (London) F.R.C.P. Assistant Physician and Lecturer on Medical Pathology, Guy's Hospital. Cloth. Pp. 152. Humphrey Milford—Oxford University Press.

tion from the duodenum. Thus, in gall bladder disease there may be subacidity or even anacidity, in the past erroneously interpreted as a true achylia gastrica. But this may be due entirely to excessive duodenal regurgitation. It is pointed out that without chloride estimations, it is impossible properly to interpret hyperchlorhydric and achlorydric reactions. When free acid is in excess, chloride estimations alone can distinguish between a positive hypersecretion and a failure in neutralization. The important conditions promoting pathologic hyperacidity are (a) excess of secretion, (b) pyloric hypertonus preventing duodenal reflux, (c) too rapid emptying. The secretion of even a normal gastric juice into a too rapidly diminishing volume would necessarily entail a steady rise in the percentage of acidity.

Ryle believes that acid in the duodenum is not as important a factor in citing closure of the pylorus as has been taught. Others have shown with simultaneous gastric and duodenal intubation that acid in the duodenum does not necessarily close the pylorus. The most rapid emptying took place at a time when the duodenal contents were unusually acid. Carlson has found that not only acid but mechanical and even alkaline stimulation of the duodenum can produce pyloric closure. Possibly gastric emptying is entirely similar to peristalsis elsewhere, stimulation at one point producing contraction or increased tension above, with relaxation below. A tonic phase in the pylorus is followed by a phase of relaxation which, especially in the later stages of a meal, allows fluid regurgitation when the next lower portion, the duodenum, becomes tonic.

The pain of gastric ulcer is evidently not due to hyperacidity. It appears to be an expression of hypertonus, excessive peristalsis, and inhibition of pyloric relaxation, all of these factors working together to increase intragastric tension. Even the feeding of hydrochloric acid in ulcer will not increase the pain, and it may persist with complete anacidity.

Hurst remarks that hunger pains develop when only a small portion of the food is still present in the stomach and the hypertonic condition, constantly present in cases of duodenal ulcer, reaches its greatest development.

Pain disappears when tonus relaxes after the introduction of food, fluid, or gas into the stomach. Ryle suggests that the beneficial effect of sodium bicarbonate may be due chiefly to the rapid evolution of gas with consequent effective relaxation of tonus. Effervescent alkalis give more relief than do noneffervescing alkalis. In the absence of free acid, alkalis fail to give relief in ulcer, not because the pylorus is incapable of relaxation but because in the absence of acid no gas is liberated.

This theory of the cause of delayed pain in gastric or duodenal ulcer will explain the development of ulcer symptoms in nervous dyspepsia. Worry, overwork, the constant go of the business man, may produce symptoms indistinguishable from those of ulcer which, however, clear up entirely with adequate rest or following a vacation. Here the primary factor was increased tonus. Ryle believes that sodium bicarbonate relieves the immediate pain of lesser curvature ulcer mainly by facilitating belching and thereby lessening tension in the proximal portion. Ryle discusses the etiology of symptoms associated with gastric dysfunction, such as flatulence, nausea, belching, etc. His discussion of the results of gastroenterostomy and the causes for persistent symptoms will be of interest to all clinicians.

Food Values[k]

THIS is a quick reference manual for nephritics and particularly diabetics who are on weighed and calculated diets. For each article the compiler has indicated the food value for different quantities. One may find at a glance the quantity of protein, fat, and carbohydrate and the caloric value of five grams, ten grams, twenty grams, thirty grams and so on up to one hundred grams. This greatly facilitates the preparation of weighed diets. All of the foods commonly used are provided for in this book.

In addition, wherever practicable the author has indicated under each food the weight in grams of the usual portion of the food as customarily served.

[k] **Food Values—For Calculating Diabetic and Nephritic Diets. Calculated from Bulletin No. 28 U. S. A. Department of Agriculture. By Louise M. Keegan, University of Chicago, Dietitian for Polyclinic Hospital, New York City. Cloth. Pp. 106. The Macmillan Company.**

In an appendix we find the usual supplementary information with regard to methods of weighing food, conversion tables, simple urinalyses, and the preparation and administration of insulin.

This book should find its greatest usefulness in the hands of the diabetic patient and the hospital dietitian.

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*A Guide for Diabetics**

LIKE all diabetic manuals this contains the usual necessary information for patients, such as description of the disease, food principles, measurement of food, principles of dietary treatment, use of insulin and its dangers, general hygienic treatment, urinalysis, food analyses, etc.

In two outstanding features it differs from the other manuals. The last chapter is devoted to home canning of food supplies. This is reminiscent of the helpful work of Sprigge and his coworkers in Duff House Papers (Oxford Press) in which home gardening for diabetics is described in detail.

But the great interest of the book of Campbell and Porter lies in its simplification of the dietary calculations to be made by the patient. After the physician has drawn up a permanent basic sample diet appropriate to his patient's needs, containing eggs, bacon, roast beef, boiled ham, cheese, and custard, the patient may then substitute an almost endless variety of foods for any amount of one or more of these basic ingredients by simply referring to a most exhaustive *table of equivalents*. No calculation is necessary after the calculation of the standard basic diet.

Defective Memory—Absentmindedness and Their Treatment†

THE first portion of this book consists in a popular presentation of the psychology of memory. The information is consistent with our psychologic knowledge.

"If I desire to recall something to mind, I must first fix it there. A thing which has not been fixed cannot be remembered. If, however, I desire to remember a thing well, I must focus on it. At this point will power becomes necessary. A man who lacks this is unable to concern himself long enough with an object to concentrate his entire attention upon it and to observe it from every angle. His observation of it will be fleeting, his fixation of it but superficial, and consequently his remembrance of it will be but short."

"Easy distraction is a great detriment to the capacity for attentive fixation." "Memory in the case of the aged cannot usually be perfect because the sense organs are dulled and no true images can be formed."

"If a person or thing is looked at carefully, examined critically from every side, and so well fixed in the mind because a perfect image of it is formed, there will be no trouble in keeping it in the memory."

Memory is of two kinds, repetitive and associative. The former is based upon constant repetition of the idea to be recalled and the latter upon its association with other ideas, surroundings, or similar situations. The best memory consists of that developed by a combination of the two.

The author establishes his idea that in most instances memory impairment is but a symptom and is associated with functional or organic disease, such as cerebral arteriosclerosis, intestinal toxemia, thyroid deficiency, deficient secretion of the other endocrine glands, constipation, infection, pathology in the nose and the like. The greater part of the volume is therefore devoted to a discussion of the treatment of these medical complications.

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*A Guide for Diabetics. By Walter R. Campbell, University of Toronto, and Mame T. Porter, Toronto General Hospital. Cloth. Pp. 259. The Williams and Wilkins Company. Baltimore, Md., 1926.

†Defective Memory—Absentmindedness and their Treatment. By Arnold Lorand, M.D. Carlsbad, Czecho-Slovakia. Author of *Old Age Deferred*. Cloth. Pp. 340. F. A. Davis Company, Philadelphia.

A supposed relationship between diseases and the type of forgetfulness is brought out. Thus, the author states that in senile dementia disturbances in the power of fixation are more pronounced, while in general paresis it is the disturbance in the power of recollection that gives the trouble. Again in neurasthenia the power of fixation is frequently affected in the highest degree, entirely as a result of the ennui and listlessness of the neurasthenic, who takes little or no interest in things about him.

In the treatment of the systemic diseases mentioned, the author follows almost without exception the generally accepted therapeutic measures. We are glad to note that Dr. Lorand's attitude on gland therapy and particularly the more recently recommended operations for rejuvenation is thoroughly conservative.

The treatment of defective memory consists in improving the patient's general physical condition. In addition to this in certain types of cases good results are claimed for thyroid therapy, ovarian therapy, by treatment of cerebral arteriosclerosis and the like. The author outlines his own plans for training the memory. As usual these are based principally upon fixation of the attention and upon the calling up of associative memory pictures.

One of the enjoyable features of the volume is its wealth of anecdote on the memory of important personages in history.

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*The Conquest of Disease**

WHEN we sigh for the good old days when men were men, all women were fair, and life was aglamour with romance, we shall do well to think of the toothless hags of forty, the decrepit roués of fifty, the open sewers running down the center of ill-paved streets, the filth, the ignorance and general suffering in all but modern times.

The influenza epidemic of 1918, occurring within our own times was to us the greatest plague which the earth has ever borne. But let us turn back the pages of history. The plague in 80 A.D. is said to have destroyed as many as 10,000 in a single day in Rome which was then a city of about 1,000,000 inhabitants. The "black death" of the fourteenth century is still without parallel. In it Cairo lost daily from 10,000 to 15,000 souls. In China more than 13,000,000 are said to have died of it. Cypress lost all of its inhabitants and ships without crews were often seen in the Mediterranean. In Avignon the Pope found it necessary to consecrate the Rhone that bodies might be thrown in without delay as the churchyards could no longer hold them.

The greatest castles, built by feudal lords, had no such convenience as a bathroom or any proper means of disposing of human filth. Excrement was thrown into the surrounding moat and it is barely possible says Rice, that the stench arising from the moat was of value in keeping away enemies. No wonder, says Rice, that heavy perfumes and smelling salts were much in demand. As a special privilege nuns of certain orders were permitted to bathe once a year, but the immodest practice was not encouraged.

Nineteen hundred and twenty-seven is a happy time in which to live. But how did it become such? Chiefly through great medical and sanitary discoveries and their practical application in public health and preventive medicine. Dr. Rice has written a most readable popular exposition on preventive medicine. It may be read with pleasure by the physician and with the greatest profit by the intelligent layman. The illustrations are plentiful, well executed, and informative. There comes first a description of the scientific conquests of transmissible disease, the cause of transmissible disease, infection and resistance, then a detailed description of those various diseases which are usually studied and discussed by epidemiologists. The last section deals with the means by which transmissible diseases are controlled, such as vital statistics, segregation, isolation and quarantine, disinfection, sanitation and public health administration.

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*The Conquest of Disease. By Thurman B. Rice, A.M., M.D., Assistant Professor of Sanitary Science, Indiana University. Illustrated. Cloth. Pp. 363. New York, The Macmillan Co. 1927.

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EDITORIALS

Chronic Appendicitis

THE early surgical knowledge of disease of the appendix consisted in recognition of the fact that some people with severe, acute pain in the right lower quadrant of the abdomen later developed abscesses in this region which could be drained surgically. With the advent of abdominal surgery the idea gradually gained acceptance that the acutely inflamed appendix was an early stage in the development of abscess and that its removal would prevent this sequel. It soon became apparent that not all cases of acute appendicitis went on to abscess formation, and the conclusion gained acceptance that the disease may be intermittent, with irregular flare-ups and intervening periods of quiescence or relative quiescence.

The fourth conception to develop was that there need not necessarily be an antecedent acute attack but that the organ may from the first be the site of low-grade chronic inflammatory processes responsible for both local and remote symptoms and organic alterations. Many and diverse ills have been attributed to chronic appendicitis.

Unfortunately far too many patients operated upon in the name of chronic appendicitis have experienced no relief from their symptoms, even though the appendix has been removed. The physician at first saw many more of these unsuccessful results than did the surgeon, but as a large percentage of the patients later required or had operations for other pathology apparently causing the same symptoms, the surgeons also gradually became aware of the prognostic disadvantages of operating merely for so-called chronic appendicitis. This led many of the more inquisitive among them to carry out extensive follow-up work on their own patients from which they were able to draw conclusions as to the ultimate benefit to be derived from operation.

So much is known of the pathology and treatment of appendicitis in general that when we see reports of symposiums on appendicitis, we conclude at once that they were presented before some county medical societies. That all is not well in our understanding of chronic appendicitis is now indicated by a symposium on this subject, presented before the section of surgery at the 1927 meeting of the British Medical Association.

Both sides of the argument were well presented. Wilfred Trotter admits the existence of chronic appendicitis without preexistent acute attacks. Such an appendix may be abnormally long, is usually abnormally fixed either by its mesentery in its proximal half or by being tied down to the posterior wall of the cecum; its caliber is not uniform but shows some dilatation, usually toward the free end, and it contains elongated, separate, more or less firm fecal masses, while its mucous membrane looks swollen and spongy. The lymph glands at the root of the appendix and in the ileocecal angle have the subglobular shape and glassy look of chronic inflammatory enlargement. The cecum is usually large, inert, and contains solid fecal material. Trotter stresses the evidence of septic absorption shown by enlarged lymphatic glands and the evidence of disturbed motor and perhaps secretory functions shown by the inability of the appendix to empty itself. Glandular enlargement is common at all ages, but especially in young adults. He suggests that it is probably as common in appendicitis as in tonsillitis but that it is often not looked for. He believes that acute lymphadenitis is a frequent event in the chronic appendicitis of children and an important cause of local symptoms. Gastric symptoms, flatulence, constipation, and sometimes diarrhea are attributed to disturbed peristaltic functions resulting in nerve irritation in the appendix.

Victor Bonney attributes the frequent operations for chronic appendicitis in women in part at least to faulty diagnosis. Three gynecologic conditions in particular are responsible for this: first, disease of the right fallopian tube; second, cyst of the right ovary particularly blood cysts (the so-called chocolate cyst), and third, retroversion of the uterus with its resultant drag on the ligaments about the cecum, always more definite than that on the looser attachment of the colon in the left side.

A. J. Walton believes that primary chronic appendicitis is a doubtful entity. He, like Willys Andrews, believes that a chronic appendicitis should never be diagnosed unless there has been at least one acute attack. The chief pathologic changes found in the so-called chronic appendix are a diffuse fibro-

sis of the appendix with obliteration of the lumen, usually commencing at the tip and steadily progressing downward, and the presence of periappendicular bands, binding the appendix to the cecum and under surface of the mesentery. He believes that these are degenerative or involutionary rather than inflammatory changes. He attributes the greater frequency of pain in the right lower quadrant in women in part to greater frequency of visceroptosis and mobile cecum. Sherren has stated that pain commencing in the right iliac fossa is not due to chronic appendicitis. Maclaren has observed that the appendix is frequently removed for chronic appendicitis with unsatisfactory results, but he distinguishes the so-called chronic appendicitis from the relapsing type. Stanton investigated a series of 100 patients who had been operated upon for chronic appendicitis and found that only 65 patients had been relieved of their symptoms.

In Walton's experience all those cases of so-called chronic appendicitis with pus in the lumen of the appendix, dense adhesions or strictures with concretion formation had had at least one previous acute attack.

In this country Robert Morris has offered a classification of five kinds of chronic appendicitis. He states that the most frequent kind is that which leads to the largest number of mistakes in prognosis and which furnishes a great group of worthless appendix operations. It is an irritative lesion belonging to the normal involution of the appendix, consisting essentially of connective-tissue fibrosis. He believes that this involutionary process occurring in the appendix in young people is a stigma of physical decline and is but a part of a group of stigmas which may be readily discovered. He speaks of the short sternum with ptosis of abdominal viscera, loose kidney, narrow costal angle, crowded teeth, and the like. Removal of the appendix does little good in this type of condition, which in general he classes as neurotic.

The other types of chronic appendicitis in his classification are, appendices showing scar tissue following an antecedent acute appendicitis, chronic infectious processes involving the cecum as well as the appendix and sometimes associated with entozoa in the appendix, lymphoid hyperplasia of the organ, and chronic congestion associated with chronic congestion of other parts of the bowel related largely to blood or lymph circulatory disturbances.

Hertzler has recently presented a pathologist's viewpoint. He is most emphatic in his conclusion that the so-called primary chronic appendix does not exist. Such changes as exfoliation of the epithelium, hemorrhages in the lumen, diminution in the number of goblet cells, moderate increase of mononuclear cells in the submucosa, increase in connective tissue in adults, widened spaces between the muscular bundles, and degrees of hyperplasia of the vessel walls, are not indicative of disease of the organ and occur far too frequently in appendices which have never given rise to any symptoms whatsoever. He has no hesitancy in declaring that a pathologic basis for chronic appendicitis does not exist. Those appendices in which changes are found to an unmistakable degree are found in persons who have had an acute attack but who no longer have complaints referable to the appendix. Hertzler's conclusions are perhaps a little overpositive and susceptible to some exceptions, but they are worthy of repetition here.

"1. Fibrotic changes in the appendix, no matter of what degree, are not attended by clinical symptoms.

"2. The anatomic structure of appendices commonly removed under the diagnosis of chronic appendicitis shows no variation from the appendices of individuals suffering from no abdominal complaint whatsoever.

"3. The minimal changes alleged to be present in cases of so-called chronic appendicitis are wholly inadequate to explain the symptoms ascribed to them considered in the light of like changes in other organs of the body.

"4. Mere alleged relief of symptoms after the removal of the appendix is not sufficient to prove that the appendix was the cause of the symptoms.

"5. The vast majority of patients so operated upon do not even claim relief of their symptoms.

"6. The symptoms alleged to be due to chronic appendicitis can be relieved by searching out the actual cause and by removing it, relieving the patient without molesting the appendix."

Heyd observed in his series that those patients in whom the appendix was removed for simple localized right-sided pain were not cured, but the patients that had appendectomy for symptoms referable to the upper abdomen and in whom there was no demonstrable pathology in either the gall bladder or the stomach were almost invariably cured. It was interesting to find that there was almost universally a history of a previous attack of pain which could be logically interpreted as an acute attack of appendicitis.

Charles L. Gibson has recently reviewed a series of cases operated upon for chronic appendicitis in the New York Hospital and compared the results for the years 1913 to 1918 with those for the interval 1919 to 1923. He is inclined to attribute the obviously better results in the second series in great part to the substitution of picric acid for iodine as a disinfectant. However, a study of his paper makes one wonder whether more careful selection of cases may not have been a more important factor. At least if all surgeons would follow the rules which he has drawn up for selection of cases for operation the results should be excellent. These rules are as follows:

"1. A comprehensive and detailed history.

"2. A complete and thorough physical examination, including all refinements of diagnosis.

"3. Exercise caution in undertaking operation on women as compared to men.

"4. Exercise caution, particularly in the more mature patients, particularly women. In this class other lesions may coexist or may be mistaken for appendicitis.

"5. Avoid the neurasthenics of any age or sex.

"6. Exercise particular restraint when there is no clear and reliable history of well-defined attacks, particularly of localized pain accompanied by nausea or vomiting.

"7. Make a good-sized incision, and, even if a frankly pathologic appendix is found, look for other possible lesions.

"8. If no obviously pathologic appendix is found, do not cease looking for other lesions until every other possibility has been exhausted; make a supplementary incision if necessary."

If we grant that there is much evidence against the so-called primary chronic appendicitis and against the justification for surgery in this type of case, the question naturally arises what is to become of the poor victim of this symptom complex? If he is to retain his appendix how may he be relieved? We must expect the answer from the internist or gastroenterologist, the neuropsychiatrist, the gynecologist, and the orthopedist. This group, except two, has attempted to formulate the answer in a symposium delivered not long ago before the Surgical Fortnightly Review, Boston.

Charles H. Lawrence presents the study of fifty cases showing symptoms of intermittent pain in the right lower quadrant, vomiting, nausea, and disturbance of function of the bowels over a period of a year or more. Twenty-one of these lost their appendices and of these twenty-one none was completely relieved by the operation. Seven obtained partial or temporary relief. The most constant symptom in the entire group was constipation. Twenty-seven showed definite cecal stasis with a more or less dilated cecum which in sixteen cases was definitely low or more or less fixed. Twelve showed stasis more or less throughout the colon, and two showed stasis in the ileum. Fifteen showed incompetent ileocecal valve, three diverticulae of the large bowel, three adhesions involving the bowel, and one patient showed a questionable duodenal ulcer on x-ray. In only one of the fifty cases was there a history of an attack that might be called acute appendicitis. The chronically inflamed appendix is probably only one evidence of a pathologic condition in the lower bowel. The condition consists of a mechanical obstruction to the proper evacuation of the intestine. Due to the inability of the intestine to empty itself properly, there results a low-grade inflammation not only of the appendix but of the cecal region and, in many cases, of a considerable portion of the large intestine. In many cases, if this low-grade inflammation is eliminated, removal of the appendix becomes unnecessary. The patient usually exhibits the picture of probable ptosis, constipation, and toxic absorption from the intestinal tract.

There is usually slight tenderness over the cecum without muscle spasm and with considerable gurgling on palpation. The cecum itself can often be felt. Pressure over the cecum often gives rise to nausea or epigastric discomfort, particularly in patients with incompetent ileocecal valve. Lawrence found that 34 per cent of his series showed headache or vertigo which was relieved when the stasis was overcome. Indican is often present in the urine.

Lawrence's outline of treatment consists first of elimination of the constipation and cecal stasis with laxatives in the beginning, if necessary, increased fluid and fruit intake, mineral oil, atropine and belladonna when indicated, a nonresidue diet with the avoidance of fats and dried foods and tonic setting-up exercises, particularly abdominal exercises. Abdominal supports should be avoided if possible. He finds that over 50 per cent of his series have been greatly improved by medical treatment.

Loring T. Swain contributes an orthopedic discussion. He attributes the attacks in this type of case to the cecum failing to empty due to diet, associated colitis, increased sag, muscle fatigue, and general exhaustion from overwork. The problem is how to make the cecum empty and so remove the cause of irritation and to increase the general vitality. The treatment adopted consists in daily cecal massage. X-ray studies have shown that manipulation of the cecum causes a mass movement of the whole lower bowel which quickly clears it of its contents in one big peristaltic push. Massage is therefore given directly after breakfast and after supper. It consists in a deep flat finger rotary movement beginning with the sigmoid and following the colon back to the cecum, the whole process taking about fifteen or twenty minutes. The massage is taken in two positions, one on the back and the other in a modified knee-chest position. The second part of the régime consists in lying down after each meal with a pillow under the shoulders to hyperextend the chest and raise the diaphragm and all the organs below it. This is followed by a face position lasting for one-half hour in order to change the position of the viscera. In order to prevent the loss of tone through sagging of the intestines when the patient is about his daily duties, all stoop-shouldered attitudes are avoided and correct posture is insisted upon. In these exercises rib-stretching and the abdominal muscle exercises play a part. Some patients are found to require the support of corsets or braces. A period of rest in bed for some weeks is requisite. Supports are discarded as correct posture is secured.

Edward L. Young, a surgeon, contributes similar ideas to the symposium. He finds that approximately one in five patients at the Massachusetts General Hospital in whom the diagnosis of urinary lithiasis was positively made had had from one to four previous abdominal operations in an attempt to relieve the condition. In the great majority the first operation had been an appendectomy.

He remarks that Deaver reports that out of 500 cases of chronic appendicitis operated upon 83.1 per cent were cured; but 418 of these had had one or more attacks of acute appendicitis previously. Coller, of the University of Michigan, found 89 per cent of 250 cases either cured or improved, but at the same time states that the prospect was not at all good without a definite acute attack in the past. He emphasizes that the fecal stream must be kept soft and all tendency to constipation avoided. Massage in the right iliac fossa while reclining in a tub of warm water is often very helpful as is also the medicine ball in certain cases. Correct posture is emphasized. In Young's discussion of the surgery of this condition, he pays much more attention to the cecum and ascending colon than to the appendix. He mentions two interesting cases. The first patient used abdominal massage for a year following operation, then tired of it and the symptoms returned. He again began massage, and they disappeared. This was repeated twice, thus establishing the causal relation beyond question. The second patient had a return of symptoms after operation. He consulted three surgeons, one of whom said he had gall bladder disease, another ulcer and a third adhesions. On postural treatment he was entirely relieved in less than three months without operation.

As Lawrence has well said, too often the appendix is only the innocent bystander in a bad neighborhood, and the surgeon an overzealous policeman who feels called upon to arrest some one, while the real culprit escapes.

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—*W. T. V.*

The Doctor and the Public

THERE is no denying the lack of information or the extent of the misinformation common to the public at large with regard to matters pertaining to health and disease.

That it is extreme at times is commonly admitted; that it is the fertile soil upon which the seeds of quackery and pseudoscientific absurdity flourish is not always sufficiently emphasized or appreciated.

We hear, nevertheless, something of the need for public education in these matters and see the application of various means and methods for the purpose.

Some, at least, of the so-called "health columns" of the daily press are of dubious value in that they are conducted carelessly, to give them the benefit of the doubt; others are the source of propaganda only, and still others fail of their purpose in some degree because it is one thing to have knowledge and still another to impart it to the other fellow. And to an equal extent is this true of the radio. Lectures and demonstrations are well enough in their way and are of value to those who attend them, but the horse cannot be forced to drink even though he be dragged to the stream.

The most fallow field for instruction is right at hand and within the reach of every doctor—his patients.

There is no physician who will not encounter the most gross ignorance and the most absurd ideas in those who consult him professionally. This is his opportunity to dispel a certain amount of ignorance and to instill—even though it be in minor degree—a certain amount of correct information. On the other hand, he may perpetuate and extend ignorance by careless, casual agreement with the patient that all his ills are due to "dyspepsia" and other similar pathologic amenities.

It is the duty of every physician to be more interested in the prevention than in the cure of disease, for this is the aim of the profession toward which every endeavor is bent. And ignorance and disease go hand in hand.

If the people were taught how to *keep* well rather than labored with in the endeavor to *get* well; if the aims, efforts, and methods of preventive and prophylactic measures were popularly understood and popularly abetted, the prevention of disease would soon assume astounding proportions, as witness

what has already been done despite prejudice and opposition. That disease can ever be entirely eradicated is, of course, impossible. There will always be sufficient ignorance, at times expressed in the form of various "antis," etc., and sufficient lack of interest which, together with natural effects and events, will suffice to continue disease as an entity. Nevertheless, a great deal can be done by persistent and consistent effort.

Nothing is more discouraging or more depressing to the scientific physician than to encounter cases of malignant disease or late syphilis, for example, when they are, to all intents and purposes, hopeless, and to realize that this might have been and could have been avoided had only the patient come for aid at a propitious time; or to realize, further, that his reason for not doing so was because of a belief, for example, that, as a popular "physical culture" magazine teaches, syphilis is curable by a milk diet and cancer by the use of diet, cancer pastes, or faith.

The doctor should always be ready, willing, and eager to seize every opportunity which presents itself to explain in *simple*, *direct*, and *understandable* phraseology the tenets of preventive medicine; to make clear why certain methods, such as vaccination, are efficacious for certain diseases and the like.

Nothing will ever be achieved by leaving it to the "other fellow."

The doctor should be the source of true and correct information, and his patients should be quick to appeal to him for this and confident of receiving it. If his own concepts and ideas are so muddled and so hazy as to render him incapable of imparting it, then he is unfit for the practice of his profession. The least he can do is to refrain from careless terminology which tends to perpetuate, if it does not inaugurate, error.

The practice of medicine is a profession, and, from certain angles, it has its business elements also, but it can never be dissociated from certain mandatory duties to the public. Its chief and greatest recompense comes at the end of the day's turmoil when, and only, if the doctor with his pipe in hand and, perhaps, some favorite book on his knee, can say, looking back over the day's work: I have done my best. I have omitted nothing which might help my most desperate case; I have done nothing which could harm the slightest one. My conscience is clear as to the patient who died.

So saying, he may face all his problems with confidence that greater nor more than this can anyone ask of him.

—*R. A. K.*

DR. A. H. SANFORD
Rochester, Minnesota
President
American Society of Clinical Pathologists

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ON TO MINNEAPOLIS—SEVENTH ANNUAL CONVENTION

American Society of Clinical Pathologists

The members of the American Society of Clinical Pathologists are looking forward with eager anticipation to the next session at Minneapolis. The program bids fair to excel in interest and scientific import the magnificent record of previous conventions.

There has been a demand on the part of members to devote some consideration to the economic aspects of the field of clinical pathology. On Friday evening, June 8, there will be a Round Table Discussion, besides consideration of these matters at our business session.

The proximity of Minneapolis to Rochester will undoubtedly bring many of our members to the convention in order that they may profit by a trip to the Mayo Clinic.

The date of the meeting has been purposely placed as near to the American Medical Association Convention as possible in order to give our members opportunity to attend the big gathering. They will also be able to take advantage of the reduced transportation rates. By carrying the program over from the week-end to Monday, the intervening Sunday can be profitably devoted to committee meetings, also the pleasant reunions among comrades in the common cause, the swapping of reminiscences of our early struggles, in the formation of new friendships and pleasant associations. The wives, too, and members of families of the Fellows will find in the convention a great opportunity both for sight-seeing and social enjoyment.

Below is a complete program containing the list of papers as approved by the Program Committee.

DR. H. J. CORPER
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Chairman, Research Committee

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DR. JOHN A. KOLMER
Philadelphia, Pennsylvania
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American Society of Clinical Pathologists

Program

Of the Seventh Annual Convention

Minneapolis, Minnesota

June 8, 9, and 11, 1928

FRIDAY MORNING, JUNE 8, 1928—9 A.M.

Call to Order.

Short Business Session.

Scientific Program

The Interpretation of the Wassermann Test. By B. Markowitz, M.D., Chicago, Illinois.

Complement Preservative—A Practical Study. By B. W. Rhamy, M.D., Fort Wayne, Indiana.

The Interpretation of Borderline Allergic Reactions. By Warren T. Vaughan, M.D., Richmond, Virginia.

The Vegetative Nervous System in Epilepsy. By A. M. P. Saunders, M.D., Chicago, Illinois.

Method for Measuring the Bactericidal Action of Whole Blood Against Gram-Positive Cocci. By William Thalhimer, M.D., and Charlotte Colwell, A.B., Milwaukee, Wisconsin.

Spectrophotometric Analysis of Blood Serum in Normal and Pathologic Conditions. Study II. By Charles Sheard, Ph.D., T. B. Magath, M.D., and A. E. Osterberg, M.D., Rochester, Minnesota.

FRIDAY AFTERNOON, JUNE 8, 1928—2 P.M.

Some Tissue Culture Studies. By F. A. Hecker, M.D., Ottumwa, Iowa.

Pathology of the Thyroid Gland. By John W. Gray, M.D., Newark, N. J.

Improvement in Technic and Results Made in Examining Microscopically by the Razor Section Method Two Thousand Malignant Tissues. By B. T. Terry, M.D., Rochester, Minnesota.

Further Observations with a New Method for Cultivating Tubercle Bacilli; A Comparison with Guinea Pig Inoculation and Petroff's Method. By H. J. Corper, M.D., and Nao Uyei, Ph.D., Denver, Colorado.

Some Observations Upon the Comparative Anatomy of Tuberculosis in Various Animals. By Herbert Fox, M.D., Philadelphia, Pennsylvania.

A System of Sputum Analysis for Acid-Fast Bacilli. By Henry C. Sweany, M.D., and Asya Stanichenko, A.B., Chicago, Illinois.

Tuberculous Endometritis. By H. L. Reinhart, M.D., and Robert C. Moore, M.Sc., Columbus, Ohio. (By invitation.)

FRIDAY EVENING, JUNE 8, 1928—7 P.M.

Round Table Discussion

The Postmortem Service at the University of Minnesota. By E. T. Bell, M.D., Minneapolis, Minnesota.

The Young Clinical Pathologist. By W. G. Gamble, Jr., M.D., Charleston, South Carolina.

The Hospital Laboratory Director. By J. J. Moore, M.D., Chicago, Ill.

The State Laboratory. By K. D. Graves, M.D., Pearisburg, Virginia.

Membership. By J. H. Black, M.D., Dallas, Texas

SATURDAY MORNING, JUNE 9, 1928—9 A.M.

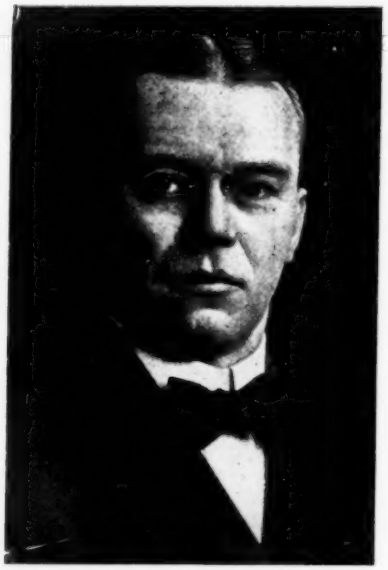
Retention of Urinary Constituents After Anastomosis of Urinary Bladder and Intestines. By F. W. Hartman, M.D., Detroit, Michigan.

The General Practitioner and the Early Diagnosis of Cancer. By Wm. Carpenter MacCarty, M.D., Rochester, Minnesota.

The Sedimentation Time of the Blood in Jaundice. By Nathan Rosenthal, M.D., M. I. Blowstein, M.D., M. Rachmilewitz, M.D., New York City.

DR. W. W. COULTER
Houston, Texas
Executive Committee

DR. HERMAN SPITZ
Nashville, Tennessee
Executive Committee



DR. F. E. SONDERN
New York City
Chairman,
Publication Committee
Executive Committee

DR. WM. G. EXTON
Newark, New Jersey
Executive Committee

DR. GEORGE IVES
St. Louis, Missouri
Chairman, Board of Censors

Studies in Sedimentation Time. By Asher Yaguda, M.D., Newark, N. J.

Some Observations on the Mechanism of the Sedimentation Rate. By O. L. Spohr, M.D., and Robert A. Moore, M.Sc., Columbus, Ohio.

A New Method of Colorimetry. By Wm. G. Exton, M.D., Newark, N. J.

The Chemistry and Cytology of Serous Fluids. By Alvin G. Foord, M.D., Guy Youngberg, Ph.D., and Vera Wetmore, B.S., Buffalo, New York.

Value of Nuclear Deviation (Schilling Classification) in Blood Examinations for Clinical Medicine. By F. W. Niehaus, M.D., Omaha, Nebraska.

SATURDAY AFTERNOON, JUNE 9, 1928—2 P.M.

A New Method for Hemoglobin Determinations. By Charles Sheard, Ph.D., and A. H. Sanford, M.D., Rochester, Minnesota.

Pertinent Facts Concerning Hemoglobin. By C. E. Roderick, M.D., Battle Creek, Michigan.

The Specificity of Bacteria to the Bacteriolytic Action of Chemicals With a Note on This Application to Chemotherapy. By Robert A. Keilty, M.D., Washington, D. C.

Results in Various Diseases from the Elimination of Foci of Infection and the Use of Vaccine Prepared from Streptococci Having Elective Localizing Power. By E. C. Rosenow, M.D., and A. C. Nickel, M.D., Rochester, Minnesota.

Further Studies on Brucella Abortus in Man. By A. S. Giordano, M.D., and Marjorie Ableson, South Bend, Indiana.

Lymphatic Leucemias and Mouth Infections. By A. S. Rubnitz, M.D., Omaha, Nebraska.

Examination of Blood for Malaria. By Leon S. Lippincott, M.D., Vicksburg, Mississippi.

Purpura Hemorrhagica with Report of Three Cases. By Oscar B. Hunter, M.D., Washington, D. C.

Estimating the Increment in Bactericidal Power of Individuals' Blood Produced by Intravenous Injection of Typhoid Vaccine. By Charlotte Colwell, A.B., and J. L. Yates, M.D. (by invitation), Milwaukee, Wisconsin. Read by title.

SATURDAY EVENING, JUNE 9, 1928—7 P.M.

Annual Banquet

Presidential Address. By A. H. Sanford, M.D., Rochester, Minnesota.

The Cults. By Wm. O'Brien, M.D., Minneapolis, Minnesota.

Greetings from the American College of Surgeons. By M. T. MacEachern M.D., Chicago, Illinois.

MONDAY, JUNE 11, 1928—9 TO 12 A.M. AND 2 TO 5 P.M.

Business Session

Call to Order.

Reading of Minutes.

Unfinished Business.

Reports of Committees:

Executive Committee—Dr. J. A. Kolmer, Chairman, Philadelphia, Pa.

Public Relations Committee—Dr. F. E. Sondern, Chairman, New York.

Committee on Exhibits—Dr. A. C. Broders, Chairman, Rochester, Minn.

Publication Committee—Dr. J. A. Kolmer, Chairman, Philadelphia.

Research Committee—Dr. H. J. Corper, Chairman, Denver, Colorado.

Committee on Registration of Technicians—Dr. Kano Ikeda, Chairman, St. Paul, Minnesota.

Committee on Blood Groups—Dr. F. W. Hartman, Chairman, Detroit.

Service Bureau Committee—Dr. R. A. Kilduffe, Chairman, Atlantic City.

Election of New Members.

New Business.

Nominations—Report of Nominating Committee.

Election of Officers.

Induction of Officers.

Adjournment.

DR. W. F. THOMSON
Beaumont, Texas
Board of Censors

DR. ROBERT A. KILDUFFE
Atlantic City, N. J.
Board of Censors
Chairman, Service Bureau Committee

DR. H. R. MILS
Tampa, Florida
Board of Censors

DR. REUBEN OTTENBERG
New York City
Board of Censors

DR. C. H. MANLOVE
Portland, Oregon
Board of Censors

DR. A. C. BRODERS
Rochester, Minnesota
Chairman, Committee on Exhibits

DR. KANO IKEDA
St. Paul, Minnesota
Chairman, Committee on Registration of Technicians

All the members of the American Society of Clinical Pathologists will mourn the untimely demise of the Secretary and one of the founders of the Society, Dr. Ward T. Burdick. His presence will be sadly missed at the next meeting. An obituary and biography will appear in a future issue of this Journal.

Dr. Sanford, our President, has asked Dr. H. J. Corper to act as Secretary-Treasurer, pro tem. until the next convention, when a successor to Dr. Burdick will be elected. Address all correspondence to the American Society of Clinical Pathologists, Children's Hospital, Denver, Colorado.

AMERICAN ASSOCIATION FOR THE STUDY OF ALLERGY

Program

MORNING SESSION—JUNE 11, 1928

1. Castor Bean Dust Asthma.
   Karl D. Figley, M.D., Toledo, Ohio.
2. Urticaria.
   Zella White Stewart, M.D., Iowa City, Iowa.
3. The Variability of Skin Reactions to Pollens.
   Harry L. Huber, M.D., Chicago, Ill.
4. Asthma in Children. Roentgen Study of the Chest. Lantern Slide Demonstration.
   M. Murray Peshkin, M.D., New York City, N. Y.
5. Allergic Bronchitis.
   George L. Waldbott, M.D., Detroit, Mich.

AFTERNOON SESSION

1. Presidential Address.
   Harry S. Bernton, M.D., Washington, D. C.
2. The Pathology of Asthma: Autopsy Reports.
   Bernard Steinberg, M.D., Toledo, Ohio.
3. Incidence and Significance of Negative Skin Tests in Pollen Asthma in Infants and Young Children.
   I. S. Kahn, M.D., San Antonio, Texas.
4. Some Causes of Therapeutic Failure in Clinical Allergy.
   Warren T. Vaughan, M.D., Richmond, Va.
5. The Mechanism of Negative Cutaneous Reactions in Hay Fever.
   Samuel Feinberg, M.D., Chicago, Ill.
6. The Value of Phosphorus and Calcium in Bronchial Asthma, Hay Fever and Allied Diseases. A Study of 150 Cases.
   Alexander Sterling, M.D., Philadelphia, Pa.
7. Comparative Pollen Counts in Various Districts of the United States.
   O. C. Durham, Indianapolis, Ind.
8. Some Unusual Manifestations of Allergy.
   Samuel J. Taub, M.D., Chicago, Ill.

MONDAY EVENING—BANQUET

Speaker of the evening:
A Critical Review of the Mechanism and Terminology of Allergy.
John A. Kolmer, M.D., Professor of Pathology, University of Pennsylvania, Pa.

MORNING SESSION—JUNE 12, 1928

1. Further Observations on the Use of Filtered Air in the Diagnosis and Treatment of Allergic Conditions.
   Milton B. Cohen, M.D., Cleveland, Ohio.
2. The Potential Asthmatic.
   F. M. Pottenger, M.D., Monrovia, California.
3. Acquisition of Human Hypersensitiveness.
   Ray M. Balyeat, M.D., Oklahoma City, Okla.

Election of officers for the ensuing year and a business meeting will be held following this session.